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JEWELL

EFFECTS OF THE HYDROGEN ION CONCENTRATION AND OXYGEN CONTENT OF WATER UPON REGENERATION AND METABOLISM IN TADPOLES.

EFFECTS OF THE HYDROGEN ION CONCENTRATION AND OXYGEN CONTENT OF WATER UPON REGENERATION AND METABOLISM IN TADPOLES

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY

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EFFECTS OF THE HYDROGEN ION CONCENTRATION AND OXYGEN CONTENT OF WATER UPON REGENERATION AND METABOLISM IN TADPOLES

INTRODUCTION

The direct influence of temperature upon the rate of development has long been recognized and has recently received considerable attention, especially from the economic entomologists who are working upon the relation of weather conditions to the time of emergence of insect pests. That other environmental factors may similarly affect development is also recognized but as yet the literature is meager.

Probably no single environmental factor is of greater importance to aquatic animals than the chemical reaction (hydrogen ion concentration) of the water. Shelford and Powers (1915) have shown that marine fishes are extremely sensitive to slight variations in the hydrogen ion concentration of the water, and Wells (1915) has shown the same to be true of fresh water fishes.

The first study of the effect of hydrogen ion concentration upon the rate of development is that of Loeb (1898). He compared the development of eggs of the sea urchin in normal sea water with those in sea water to which 2 c.c. $^{n}/10$ NaOH per 100 c.c. had been added. At first no difference was noted but after the thirty-two to sixty-four cell stages, it was evident the eggs in the alkaline solution had developed more rapidly. The embryos in normal water were still blastulae when those in the alkaline water were plutei, and at the end of forty-eight hours, though all were plutei, the ones in alkaline water were larger and farther developed. One, two

or three c.c. $^n/10$ HCl per 100 c.c. was found to retard development.

Working upon the eggs of fundulus in fresh water, Loeb found that 4 c.c. $^n/10$ NaOH per 100 c.c. caused them to hatch faster. More than 6 c.c. retarded development. 12 c.c. allowed only a few of the eggs to hatch, while 15 c.c. resulted in failure of any to hatch. 2 c.c. of $^n/10$ HCl killed all of the eggs, while 1 c.c. killed most of them. Loeb attributed these results to an increase of oxidation by bases and a decrease by acids.

Later Loeb (1914) decided that the acceleration of development of the sea urchin eggs in water to which NaOH had been added did not appear until the thirty-two to sixty-four cell state because at first the concentration was too high, but after a few hours the beneficial effects appeared as the results of neutralizing the acids produced by the embryos during development. These conclusions were based upon a comparison of the rate of regeneration and growth of tubularians in a solution of NaCl, KCl, $CaCl_2$ and $MgCl_2$ in the proportions in which they occur in sea water, and in the same solution to which NaOH, $NaHCO_3$ or Na_2 HPO_4 had been added. Although the growth was invariably better when a base had been added, as the effects of the weaker bases were better than those of the NaOH, Loeb concluded that the results were not due to the free OH ions but to the ability of the base to neutralize the acids produced by the tubularian. Loeb and Wastney (1913 a and b) have shown that bases increase the rate of oxygen consumption in both the unfertilized and fertilized eggs of strongylocentrotus purpuratus but that in fertilized eggs this increase in oxygen consumption is accomplished only by concentrations which cause a suppression of the phenomena of development.

Moore, Roaf and Whitley (1905) worked upon the effects of alkalies and acids and of alkaline and acid salts upon growth and cell division in the fertilized eggs of Echinus esculentus. The bases used were NaOH, KOH, $Ca(OH)2$

and NH_4OH. The acids were HCl, CH_3COOH and CO_2 and the salts Na_2CO_3, $NaHCO_3$, Na_2HPO_4 and NaH_2PO_4. These workers found that bases, except NH_4OH, give acceleration of development in low concentrations, while higher concentrations check development and finally kill. Acids all inhibit. The primary factors affecting the rate of growth appear to be the variations in concentration of hydrogen and hydroxyl ions. Thus all of the caustic alkalies are of approximately equal power and there is little or no action of the cations. But in the case of the phosphates, where the hydrogen-hydroxyl ion concentrations are comparatively low, there seems in addition to be a specific factor. The extreme limits of variation of hydrogen and hydroxyl ion concentrations within which growth is possible are shown to be very narrow, .0015 M. caustic alkali or .001 M. acid practically stopping all development. Whitley (1905) showed the same relationship between hydrogen-hydroxyl ion concentration and development to hold for the eggs of Pleuronctes platessa. He gives three reasons why alkali is less harmful than acid:

1. Alkali added to sea water is immediately thrown out as insoluble hydrates or carbonates.

2. Alkali is constantly used up to neutralize CO_2 produced by the animal.

3. Loeb has shown a low quantity of alkali increases the permeability to O_2. This may increase the resistence of the egg.

Finally Grace Medes (1918) has shown that changes in the composition of the sea water by concentration, dilution or addition of acids, bases or salts in non-lethal concentrations cause a retardation of development of the eggs of arbacia punctulata.

Excepting for the experiments of Loeb in 1898 on Fundulus eggs, nearly all of the work so far done on the effects of bases and acids on development has been done upon marine animals. Many of the most serious

difficulties in such investigations arise as the result of the chemical composition of sea water and may be largely eliminated by the use of fresh water forms. Thus Haas (1916) has shown that the addition of hydroxids to sea water results in a proportionate increase in OH ion concentration only after all of the Ca and Mg have been precipitated out in the form of their basic carbonates.

This discovery is of twofold importance for it means in the first place that much of the base added to the sea water is immediately thrown out, and in the second place, the disturbance of the balance between the salts of Na, K, Ca and Mg, which Loeb (1903) has shown is exceedingly harmful to marine animals.

In working with acids too, the use of sea water presents certain difficulties,for any strong acid added to sea water will immediately form salts with the liberation of CO_2. Hence no comparison of the effects of the various mineral acids is possible,as the acidity exisiting in the sea water is due to H_2CO_3. For these reasons, and also to see whether fresh-water animals are adjusted to a different H ion optimum than marine organisms, the author at first wished to undertake a study of the effects of bases and acids upon development and growth of some fresh water form. Since, however, there were no eggs or young larvae available at the time of year when this investigation must be done, it was decided to use regeneration which in many ways resembles original growth. This seemed further desirable because no such studies of regeneration have as yet been made and it is probable that comparative studies of regeneration and growth under varying environmental conditions might throw much light upon the extent of the similarity or dissimilarity of the two processes. What is true of the effects of hydrogen ion concentration is also true of the effect of oxygen on regeneration. As yet no experimental studies are known to have been made on regeneration in varying concentrations of oxygen. As rapid metabolism and rapid growth are usually associated, the metabolism experiments were undertaken to see whether any correlation could be traced between the rate of

regeneration of a part and the metabolism of the animal as a whole and whether substances stimulating to oxygen metabolism are, as Whitley supposed, beneficial to the organism. In this work the author has been concerned only with the extrinsic factors of regeneration. The intrinsic factors have been considered only as they arose as necessary corollaries to the work on environmental effects.

Materials

The tadpoles studied were larvae of Rana climata (Daw) collected from a spring-fed marsh near Muncie, Illinois. Three collecting trips were made, dated October 9, 1917, March 5th and 24th, 1918. This supply was supplemented during the middle of the winter by a few tadpoles purchased from a local dealer. All tadpoles were identified by means of key to larval amphibia Wright (1914). The stock of tadpoles was kept in the greenhouse in an albarine tank supplied with running water to a depth of about 6 inches. The food supplied was filamentous green algae. All tadpoles were kept fasting during experiments.

Measurements of regeneration were made by means of a caliper and metal C.G.S. ruler,obtained from Spencer Lense Company. The tail was the only organ experimented upon. In making averages there was no elimination of individuals,excepting in one or two instances,and there note is made of the fact and the aberrant individual included in the table.

APPARATUS AND METHODS

The oxygen free water was obtained from the water boiling apparatus in the vivarium at the University of Illinois which has been described by Shelford (1918). This apparatus consists primarily of a boiler in which the water is boiled by means of high pressure steam until practically all of the gases are driven out and from which it passes into a tank covered by a hood where boiling is continued until the water is freed of gases. The flow of water into the boiler is regulated by a float cork. The oxygen free water is withdrawn from the hooded tank, passes through cooling pipes in the room below where it is used. As it comes from the pipes it contains no oxygen (Winkler method) and is strongly alkaline, due to the CO_2 having been expelled by long boiling and much of the bicarbonate having been converted into carbonates. A series of different concentrations of oxygen was obtained by syphoning the water through a series of half-gallon mason jars (fig.1) through alternate ones of which air was bubbled. The water from the last of these was siphoned into a five-inch beaker so that the level of water in the jars could not fall below five inches. It was found by titration that the O_2 concentration varied greatly in the different parts of a jar through which a stream of air was passing, but was relatively constant except at the surface in a jar through which the water was being siphoned but no air was passing; consequently the jars containing the experimental animals were alternated with those which were being aerated, with the result that each experiment jar contained a little more O_2 than the one preceding.

The flow of air through the water was regulated by passing it through capillary tubes drawn to a fine point and the air pressure was kept relatively constant by means of a mercury manometer made by bending a U at one

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

end of a 5 ft. glass tube and attaching it at the end of the air line. Mercury was then poured into the tube until a pressure was reached at which air would bubble through the jars at the desired rate.

Daily O_2 determinations were made on the water from each jar by the Winkler method. Samples for these determinations were collected by introducing a Powers' sampling bottle (Powers 1918) between two jars and allowing the water to siphon through it fifteen minutes.

As has been mentioned above, the oxygen-free water from the boiler is strongly alkaline. Where neutral water was desired, this was remedied by means of the acidulator (upper left hand of fig. 1) which consists of a twelve-liter aspirated bottle placed in a large earthenware milk pan, with the upper opening corked securely. The acid in the pan will stand at the level of the top of the lower opening in the bottle, thus giving a constant pressure in the siphon which is drawn to a capillary tube at the distal end. The flow of the acid is regulated by the size of the capillary tube. The acid drops from the siphon into the separatory funnel, through which it passes to the bottom of the ten-liter mixing bottle, where it is thoroughly mixed with the water before entering the experimental jars; n/10 H_2SO_4 was used. The acidity of the water could be regulated by counting the number of drops of acid per minute and regulating the flow of water through the mixing bottle accordingly.

The Distilled Water.

In some preliminary experiments, ordinarily distilled water obtained from the Department of Chemistry was used. This water is condensed in a copper condenser and passes through blocked in tin pipes. It was found not only to be very toxic to tadpoles but to vary in its effect from day to day so that tadpoles might live several days in water taken from one bottle and die in a few hours in water taken from another. The same water redistilled in glass

supported the life of tadpoles of Bufo lentiginosus Le Conte through metamorphosis. These results are in accord with the findings of Bullot (1914) and Powers (1918 b) who attributes the toxic effect of ordinary distilled water to traces of colloidal copper taken up from the condenser. Powers had shown that a goldfish may live fifty-one days in water redistilled in glass but succumbs quickly if the minutest traces of colloidal copper be added to the water.

The water for all of the experiments was condensed in a six-foot Jena glass condenser attached to the second tank of the water-boiling apparatus mentioned above. It was then aerated for 24 hours by a stream of air previously passed through sulphuric acid and calcium hydroxid wash bottles. This distilled water was neutral to Rosalic acid and neutral red. An analysis of the water kindly made by the water analyst of the State Water Survey was as follows:

Physical examination	
Turbidity	0
Color	5
Odor	2 V
Residue on evaporation	
Total solids	38 to 42 parts per million
Alkalinity as calcium carbonate	
Methyl orange	0 to 0 parts per million
Chlorides	
As sodium chloride	0 to 0 parts per million
Ammonia nitrogen	4 to 7 parts per million
Albuminoid ammonia	0.060 to 0.102 parts per million

A common shiner (Notropus blennius) lived in this water (changed weekly) from October 18, 1917 to February 17, 1918, or a total of one hundred twenty-two days. As, during this time, the fish decreased notably in size, its death

might be attributed to starvation rather than to the toxicity of the water. Powers (unpublished) has shown that two goldfishes lived in this water ninety-five and ninety-nine days, whereas in the chemistry distilled water, the length of life was three hundred fifty-two and five hundred ninety-seven minutes.

All chemicals used were either Marks' or Baker's analyzed chemicals. The acids and bases were standardized at $n/100$, except H_3PO_4, Na_2CO_3, $NaHCO_3$, which were $^1/100$ mol.

For the determination of CO_2 production, 1 pt clamp-top fruit jars were used. The capacity of one of these jars is about 500 c.c. 400 c.c. of water or the solution under investigation was put in a jar, then a test tube containing 10 c.c. $n/50$ $Ba(OH)_2$ was inserted so that the top of the test tube stood above the level of the water, the rubber ring was adjusted, the tadpole put in, and the lid clamped down. A stronger solution of $Ba(OH)_2$ was used where acid had been added to the water or where more CO_2 was expected. To correct for the CO_2 in the water and in the air above, a control jar was prepared at the same time and in precisely the same manner, except that no tadpole was put in it, and placed beside the experiment. At intervals, usually 24 hours, the $Ba(OH)_2$ was mixed with the water by inverting and rotating the jar. The tadpole was quickly removed, and the excess $Ba(OH)_2$ titrated with $n/100$ H_2SO_4, using Phenolpthalein as indicator. The difference in the amount of acid required to neutralize the experiment and the control was recorded as the amount of CO_2 (as $n/100$ H_2CO_3) produced by the tadpole during the given time.

The hydrogen ion concentration was determined by the method of McClendon (1916). The solution to be tested was placed in a test tube 1 cm. bore and 1/10 c.c. of the indicator solution added. It was then compared with the color chart by looking down into the tube against a white background. The indicators used were Butter yellow (Dimethyl amido azobenzene) methyl orange,

4 Br-phenol - S - pthalein (Brom Phenol blue) Methyl red, Paranitro phenol, Neutral red, 2 Br-thymol - S - pthälein (Brom thymol blue) Thymol - S - pthalein (Thymol blue), Phenolpthalein and Thymolpthalein.

Solutions of Methyl Violet and Tropavlin O O (Orange IV) were also prepared but none of the solutions used were so acid as to come in their range. On the other hand, the basic solutions went beyond the range of this color chart so the hydrogen ion concentration of only the more dilute solutions could be determined.

PRESENTATION OF DATA

Before beginning the experiments on regeneration, a few tests were made to determine approximately the concentrations to use. The first, a tadpole was put into 1 liter of n/100 solutions (except H_3PO_4 0.01 mol) of each of the bases and acids to be tested and the length of life noted as follows:

Base	KOH	NaOH	$Ba(OH)_2$	$Ca(OH)_2$	NH_4OH
Length of life of tadpole in minutes	50	65	65	60	35
Acid	HNO_3	HCl	H_2SO_4	H_3PO_4	CH_3COOH
Length of life in minutes	70	120	125	135	125

Two controls were run. One, distilled water from the metal still; This water was used in making up the solutions, and the other to eliminate the possibility of osmotic pressure having produced the effect, a solution consisting of 37 parts 0.01 mol. KH_2PO_4 and 63 parts 0.01 mol. Na_2HPO_4 which is the exact neutrality buffer solution of Levy, Rowntree and Mariott (1915) diluted to 0.01 mol. In the former the tadpole lived five days; in the latter thirty-eight days.

The second series was in .001 normal solutions (except H_3PO_4 which was .001 mol.) and gave the following results:

Base	KOH	$NaOH$	$Ca(OH)_2$	$Ba(OH)_2$	NH_4OH	Na_2CO_3
Length of life in days	31	5	30	1½	1/6	6

Acid	HNO_3	HCl	H_2SO_4	H_3PO_4	CH_3COOH
Length of life in days	1+	2-	5	8	7

As the tables show, the toxicity of NH_4OH is altogether out of proportion to its strength as a base. For this reason, as the specific toxicities of the various ions was not within the scope of the investigation, it was decided to use only the metallic bases and mineral acids.

Tables 1 and 2, experiment I; and 3 and 4, experiment II; give the regeneration of individual tadpoles in KOH and $Ca(OH)_2$ respectively. The curves of growth are shown in figures 2, 3, and 4. These experiments were carried out in finger bowls, each containing two tadpoles (one of each size) in the amount of base indicated in the table made up to 200 c.c. with distilled water. The solutions were changed daily throughout the experiment. In plotting the curves, the per cent regenerated (taking the amount removed as 100%) was used instead of the actual length regenerated, because as Zeleny (1917) has shown, "within wide limits the length regenerated in the tail of an amphibian larva is directly proportional to the length removed."

The large range of low concentrations was used to see if there might be any acceleration to regeneration by low concentrators, such as was found for development of sea urchin eggs by Loeb (1898) and Moore, Roaf, and Whitley (1905). As the growth curves show, no such acceleration appeared. In fact, the addition of bases produces no appreciable effect until 15 c.c. have been added, giving a .0075 normal base in which concentration a marked retardation of regeneration appears in both the KOH and $Ca(OH)_2$.

A comparison of tables 1 and 2, and 3 and 4, brings out another interesting point: That the larger tadpoles (tables 1 and 3) show a marked retardation of regeneration in 15 and 20 c.c. of the bases, while the smaller tadpoles (tables 2 and 4) are killed by concentrations which produce much less retardation of regeneration. That while the normal regeneration of the smaller tadpoles is much faster than that of the larger ones, the effect of any given concentration of base on regeneration is about the same for both sizes, being, if anything, more marked in the larger individual while the effect upon the animal as a whole, as shown by the length of life, is greater in case of the smaller individual. From this we may believe that while regeneration is affected by the same agents as general metabolism, the two vary independently and that the threshold of toxicity (Powers 1918 b) of a substance to the regenerating tissues may be above or below the threshold of toxicity to the organisms as a whole. This subject of the effect of size upon Regeneration and Metabolism will be more fully discussed later.

The next series of experiments (Tables 5,5a and 6. Figures 5, 6, 7 and 8) was run in order to determine whether the effect of bases upon regeneration was due to the action of the hydroxyl ions or whether the kations or osmotic pressure played an important part. 0.01 mol. solutions of Na_2CO_3 and $NaHCO_3$ were used. The number of c.c. of the solution used is given in the first column. This was made up to two hundred c.c. with distilled water. The experiments were carried on in finger bowls, each containing four tadpoles, and the water was changed alternate days.

In order to assure a uniformity of size of tadpoles in the various solutions, the eighty tadpoles used were first divided into four lots, each lot containing tadpoles of a uniform size. One tadpole from each of the four lots was then put into each of the solutions. This method for obtaining uniformity of size was used in all succeeding experiments where a large number

of animals was involved. The first, seventh, and fifteenth dishes were distilled water controls. In figures 6, 7 and 8 the 0 line, or curve of growth of the controls was plotted from the averages of these three.

If osmotic pressure is the important factor in the concentrations used, retardation of regeneration should be the same, or very nearly the same, in equi-molecular solutions of Na_2CO_3 and $NaHCO_3$. If the Na-ion is the important factor, thirty, sixty, ninety and one hundred twenty c.c. of $NaHCO_3$ should have approximately the same effects as fifteen, thirty, fourty-five and sixty c.c. of Na_2CO_3 respectively, while thirty c.c. each of Na_2CO_3 and $NaHCO_3$ should have the same effect as forty-five c.c. Na_2CO_3.

Tables 5,5a and 6 show that this is not the case. Table 5 and Fig. 5 show a progressive and rapid decrease in regeneration in increasing concentrations of Na_2CO_3. The tadpoles in 60 and 75 c.c. died in four and three days respectively without having undergone any regeneration. Table 5ashows no marked decrease in regeneration in increasing concentrations of $NaHCO_3$ until a .006 mol. solution (120 c.c. in 200) is reached, although the control gives the best regeneration. The low percentage of regeneration of the tadpoles in 30 c.c. must be attributed to chance as those in both the higher and lower concentrations showed better regeneration.

The curves of regeneration shown in figures 6, 7 and 8 bring out more clearly the difference in effect upon regeneration of Na_2CO_3 and $NaHO_3$. Thus while 15 c.c. Na_2CO_3 and 45 c.c. $NaHCO_3$ (Fig. 6) depress regeneration but little more than either of the salts alone, 45 c.c. Na_2CO_3 and 15 c.c. $NaHCO_3$ (Fig. 8) give practically the same depression as 45 c.c. Na_2CO_3 alone, which is incomparably greater than the depression caused by 45 c.c. $NaHCO_3$ alone.

Since there is this great difference in depression of regeneration between equi-molecular solutions of the carbonate and bicarbonate, and since

moreover the bacisity of the bicarbonate (P.H. given in last two columns table 5a) is sufficient to account for the slight depression in regeneration caused by them as compared to the control, it may safely be concluded that the depression in regeneration caused by bases is due to the hydroxyl ions. This conclusion seems the more justifiable in view of the fact that the concentrations of salts used in the above experiments are three to five times as great as the concentrations of the hydroxids used.

Carbon Dioxide Production and Regeneration in Bases.

As basic media are generally known to increase oxygen metabolism, it was thought desirable to try a few experiments to see whether the decrease in regeneration could in any way be correlated with the increase in carbon dioxide production. The methods employed have already been described.

Table 7, experiment 4, gives the CO_2 production in KOH of six sets of four tadpoles each. Table 8 gives the regeneration of the same tadpoles. Those in the higher concentrations (30, 35 and 40 c.c. KOH per 400 c.c.) died without regeneration. Fig. 9 (drawn from table 7) represents graphically the increase in CO_2 production with increased concentration of base and gives a curve almost identical with the curve shown in Fig. 10, which was drawn from the data of Loeb and Wastney (1913 b) for the increase of oxygen consumption in increasing concentrations of bases.

Fig. 11 gives the curves of growth for the three sets of tadpoles which survived. It shows clearly the decrease both in the rate of regeneration and the total amount regenerated in increasing concentrations.

Fig. 12 (drawn from the data of tables 7 and 8) compares the effect of the varying concentrations of KOH upon the rate of regeneration taken as time necessary to regenerate 20, 30 and 35% of the amount removed. The dotted line represents the CO_2 per gram per day produced by the same tadpoles.

It will be noted that the rate of regeneration curves and the carbon dioxide curve are not parallel. This, however, would not be expected as the normal carbon dioxide production is a straight line and the normal rate of regeneration is a curve, progressing more slowly as regeneration nears completion.

It is of interest to note that the curve of regeneration for the earliest stage shown (20% of the amount removed) corresponds more nearly to the curve of carbon dioxide production than does the curve for the second stage (30% of the amount removed) and this in turn shows more similarity than the curve for the third state (35% of the amount removed). This would suggest that at the very beginning of the process of regeneration the retardation of regeneration corresponds with the acceleration of oxygen metabolism but that later the two processes diverge progressively, due to the additional inhibition to regeneration afforded by the presence of the regenerated tissue.

Tables 9 and 10, experiment five, give the carbon dioxide production and regeneration of another series of tadpoles in KOH. In this case the normal CO_2 production of each set of tadpoles was first determined and used as a basis for comparison of the CO_2 production under experimental conditions. In graphing the CO_2 production (Fig. 14) no. 6 was omitted as these tadpoles had been injured by accidental mixing of the $Ba(OH)_2$ used to absorb the CO_2 from the air above the water (Description of apparatus) with the water in which the tadpoles were.

Tables 11 and 12 give the results of a similar series of experiments using $Ca(OH)_2$ as the base, and here again one set (No. 4) was spoiled by accidental contamination of the solution with $Ba(OH)_2$. Figures 13 and 15 are the curves of regeneration in KOH and CaOH respectively; figures 14 and 16 show the rate of regeneration as time necessary to regenerate a given per cent of the amount removed, and the CO_2 production. While these curves are not

comparable, the regeneration curves being plotted with ordinate as concentration and abscissa as time, and the CO_2 curves being plotted with ordinate as concentration and abascissa as c.c. CO_2 as 0.01 NH_2CO_3, still they do serve to show that as CO_2 production is increased, regeneration is decreased progressively.

Child (1913) has shown that for planaria the rate of regeneration is proportional to the rate of metabolism and that consequently younger or smaller worms regenerate more rapidly than the larger ones. In tadpoles, as has already been pointed out, the rate of regeneration is greater in the smaller individuals. The fact, then, that a decrease in regeneration is correlated with a rise in carbon dioxide production in basic media must be regarded as showing that the increased oxygen metabolism due to bases is a destructive metabolism and not indicative of any stimulation of the normal functions of the organism.

It will be noted that in both of the above experiments (Fig.13 and 15) the regeneration of the tadpoles in water containing ten cubic centimeters of the base made up to 400 was better than that of the controls. It will also be seen by examination of the data (tables 9 and 10) that while the initial hydrogen ion concentration of these solutions is decidedly on the basic side of neutrality (P.H. about 9), that by the time the solution was changed, it was slightly acid (P.H. about 6.8). For this reason another series of experiments was planned, using the same concentrations, but a larger volume in proportion to the size of the tadpoles and changing the solution oftener. Two parallel series were run, each containing five small tadpoles in one liter of distilled water, .00025 N. NaOH and .0005 N. NaOH(25 c.c. and 50 c.c. 0.01 N. per liter). The experiments were carried out in two-quart mason jars kept tightly sealed and containing test tubes of $Ba(OH)_2$ solution to absorb the CO_2 from the air above the water. The results of this experiment are given in table 13. Due

to the CO_2 produced by the tadpoles the distilled water became acid and the NaOH solution less basic. However, it was no longer neutralized. The retardation to regeneration (Fig. 17) is very marked in 25 c.c. NaOH per liter while 50 c.c. per liter proved fatal in two days.

These results are in accord with the suggestion of Loeb (1904) that the beneficial effect of the addition of a base is due, not to the presence of hydroxyl ions, but to the neutralization of acids produced by the animal, and show that a P H between 8 and 9 is markedly detrimental.

Regeneration in Acids

As the previous experiments show, neutrality is more favorable for regeneration in tadpoles than bacisity. Is the same thing true of acidity? A complete series from acids, almost certain to have detrimental effects to bases known to be harmful, was formed by using 25, 20, 15, 10 and 5 c.c. of 0.01 mol. H_3PO_4 and 5, 10, 12.5, 15, 17.5 and 20 c.c. of 0.01 N NaOH made up to 200 c.c. with 5 c.c. 0.01 mol. neutral Na_2HPO_4 and KH_2PO_4 mixture as a neutral control as well as the usual distilled water controls. The solutions were changed daily. It was hoped in this way to locate approximately the optimum hydrogen ion concentration for regeneration.

The results are given in tables 14 and 14a (Exp. 8). The results for H_3PO_4 (table 14) are not very conclusive, as the rate of regeneration (Fig. 18) is about the same for all concentrations which are not fatal. The total amount regenerated is, however, greater in the three controls. The neutral phosphate control lies midway between the two distilled water controls, showing that the presence of Na, K and PO_4 ions in the small amount employed is neither beneficial nor detrimental.

The results of the NaOH series (table 14a) are definite, showing a progressive decrease in both the rate of regeneration and the amount regenerated, as the concentration of base is increased. While the curve of growth

of tadpoles in 5 c.c. lies above the controls, the hydrogen ion determinations show that this solution is not only neutralized but becomes slightly acid within twenty-four hours, and it has already been shown (Experiment 7) that this same concentration of base is distinctly harmful if the volume is sufficient to prevent its being neutralized. Fig. 20 gives the time necessary to regenerate 25, 30 and 35% of the amount removed.

The next series of experiments (9, 10, 11 and 12) involving 124 tadpoles was made in order to compare the effects upon regeneration of the various acids. The experiments were carried on in finger bowls containing 200 c.c. of the solution. Four tadpoles were placed in each dish and the solutions were changed alternate days. The acids used were 0.01 N HNO_3, HBr and H_2SO_4 and 0.01 mol. H_3PO_4. Tables 15, 16, 17 and 18 respectively give the results of the experiments. The tadpoles in the HNO_3 series were slightly smaller than those in the H_2SO_4 and H_3PO_4 series, those in HBr were somewhat larger.

A comparison of the tables or figures (21, 22, 23 and 24) show that in HNO_3, HBr and H_2SO_4 the retardation of regeneration is gradual and comparatively slight in concentrations up to 12.5 c.c. (.000625 N). As the concentrations rise above this, the retardation to metabolism increases very rapidly, the order of toxicity of the acids being HNO_3, HBr, H_2SO_4. This might be explained on the ground that in the lower concentrations the acids are so nearly completely ionized as to have about the same effects, but as the concentration is increased the concentration of hydrogen ions, and consequently the toxicity increases more rapidly in HNO_3 than in HBr and in HBr than in H_2SO_4. The data is, however, insufficient to warrant any conclusions. In H_3PO_4 (Fig. 24) there was no such marked retardation of regeneration in the concentrations used although the decrease in the total amount regenerated was equally pronounced. The rate of regeneration proceeded at very nearly the

normal rate up to a certain point and was then discontinued abruptly. The H_3PO_4 series of experiment 8 (table 13, fig.18) gave similar characteristic curves, but why the form of the H_3PO_4 curve should differ from that of the other acids is not explained. Twelve and one-half c.c. 0.01 N HBr or H_2SO_4 gives about the same initial hydrogen ion concentration as twenty-five c.c. 0.01 mol. H_3PO_4 because of its greater ionization, but by the end of twenty-four hours the hydrogen ion concentration is much lower. This is because the total number of hydrogen ions which must be replaced before the solution will be neutral is greater in the H_3PO_4 , consequently it is neutralized more slowly. Thus the tadpoles in the stronger acids are in a medium of higher hydrogen ion concentration each time the solution is changed but of lower hydrogen ion concentration before it is changed again than those in the H_3PO_4, that is, they live under a more variable environment. Further experiments will be needed to show whether this is the cause of the difference in the regeneration curves or whether it is caused by some specific action of the H_3PO_4.

The tadpoles marked (T) (Tables 15, 16 and 17) were transferred to neutral distilled water to see whether normal regeneration could take place after the initial stunting with acids. Their subsequent regeneration is, of course, not added in with the other tadpoles of the group in determining the per cent of regeneration. Tadpoles transferred during the first days of the experiment underwent more regeneration than those left in the solutions but their regeneration was far below normal. At the close of the experiment all survivors were transferred to distilled water but none of them underwent further regeneration, showing that the effect of acids is not merely a retardation but a permanent inhibition to regeneration.

The curves in figure 25 represent the time required to regenerate 22.5% of the amount removed in HNO_3 and 25% of the amount removed in HBr,

H_2SO_4 and H_3PO_4. The dotted lines represent the actual experimental data, the solid lines the theoretical curve. Excepting in the case of H_3PO_4, these lines coincide very closely.

A comparison of the curves for HNO_3, HBr and H_2SO_4 with the curves for carbon dioxide production in bases (Fig. 9) for oxygen consumption in bases (Fig. 10) and for regeneration in bases (Fig. 7) shows that they are all similar. These curves are also similar to the metabolism curves drawn by Powers (1918 b) from the data of Loeb and Miss Hymen for oxygen consumption in increasing amounts of anaesthetics. On the other hand, they are the opposite of the curves of Krogh (1914 a and b) for increase in the rate of development at increased temperatures, the toxicity curves of Powers (1918 b) for the decrease in length of life with increase in concentration of toxic substances and of the curve for the increase in regeneration in increasing amounts of oxygen (Fig. 38) to be discussed later. This would suggest that whether the criterion chosen be time required to complete a given stage in development (Krogh 1914 a and b), time required to regenerate a given per cent of the amount removed, length of life (Powers 1918 b), or rate of oxygen metabolism, the effect of temperature (Krogh 1914 a and b) hydrogen ion concentration, insufficient oxygen and toxic substances (Powers 1918 b) follows the same general laws. That these various functions, - development, oxygen metabolism, regeneration, and length of life - vary independently and bear no causal relations toward one another is shown by the facts that an animal may be killed before it has begun to regenerate in a medium in which another may survive and undergo considerable regeneration, while a marked change of CO_2 production may be produced by a medium which has apparently no effect upon regeneration. This would indicate that the threshold of toxicity of any given environmental agent, as well as the velocity at which it becomes toxic, varies for the different functions of the organism. However, that any environmental factor which

affects one of these functions will, if continued for a sufficient time and intensity affect all of the functions similarly and in the same direction is suggested by the similarity of the curves. The only apparent exception is the increase in CO_2 production, due to concentrations of bases which caused retardation of regeneration, and death. However, as has been pointed out, this increase in oxygen metabolism may be regarded as a destructive metabolism and comparable to death rather than to growth or development.

Figures 26, 27 and 28 are curves giving the time necessary to complete the regeneration of various percentages of the amounts removed in HNO_3, HBr and H_2SO_4 respectively. A comparison of these curves with the curves for regeneration in bases of various percentages of the amounts removed (Figures 12, 14, 16 and 20) shows that while in some cases (Figures 14 and 20) the regeneration of tadpoles in the higher concentrations of bases seems to follow no definite plan, that in general the curves are similar. In any given series all of the curves of regeneration - which represent different stages in regeneration of the same animals - are confocal. This shows that while the rate of regeneration at different stages is different, the per cent of retardation as compared to the normal, due to the acid or base, is the same for all stages, or that for each concentration the relative velocities for the different stages are practically the same. This is similar to the findings of Krogh (1914a) in his work on the development of frogs' eggs at various temperatures.

Carbon Dioxide Production and Regeneration in Acids.

The next series (Experiments 13 and 14) was undertaken to see whether any correlation could be found between the regeneration and carbon dioxide production in acids. Ten sets of four tadpoles each of as nearly the same size as possible were used. The normal carbon dioxide production in distilled water for a period of three days was determined after a two-day fast

to allow the alimentary tract to become empty.

The first and tenth sets were then kept in distilled water as controls and the other eight put in 10, 20, 25 and 30 c.c. of HCl and HBr made up to 400 c.c. with distilled water. The carbon dioxide production, whether expressed as grams per day or as per cent of normal, decreased progressively with the increase in concentration of acid, except in case of the tadpoles in 30 c.c. of HCl (Table 19, Fig. 29) which showed a decided rise. The survivors were allowed to regenerate in the same concentrations of acids. Table 20 gives the results. The HCl series show a progressive decrease in regeneration (Fig. 30). In the HBr series the tadpoles in 20 c.c. regenerated more rapidly than those in 10 c.c. after the seventh day. This is probably attributable to chance as the tadpoles in 25 c.c. showed greatly-decreased regeneration and survived only eight days. Figure 31 gives the rate of regeneration of the HCl series as time necessary to regenerate 25, 30 and 32.5% of the amounts removed, and the CO_2 production of the same individuals as c.c. H_2CO_3 per gram of tadpole per day. It is probable that per cent of normal CO_2 production (Fig. 29) is the more logical way of representing the effect of an environmental factor upon metabolism. Here again (Fig. 31) the curves for various per cents of regeneration are confocal, showing that for each concentration the relative velocities of the different stages are the same.

Tables 21 and 22 give the results of an experiment in which CO_2 determinations were made daily throughout the period of regeneration. Four rather large tadpoles were used, care being taken to have them as nearly alike as possible. The experiment was begun at an almost constant temperature of thirteen degrees centigrade, but later was placed at room temperature in order to hasten regeneration. The effect of the acid in decreasing both the CO_2 production and the regeneration (Table 22, Fig. 32) is pronounced.

Figure 33 shows graphically the average daily CO_2 production, the per cent of normal CO_2 production, the total amount regenerated as per cent of the amount removed, and the time required to regenerate 7.5% of the amount removed. While these curves are in no wise comparable, they serve to bring out more clearly the progressive reduction in rate and amount of regeneration and in carbon dioxide production due to increasing acidity of the medium.

Relation of Size to Carbon Dioxide Production and Regeneration.

It has already been mentioned in connection with experiments one and two that there is a difference between large and small tadpoles in the relation of retardation of regeneration and length of life in various concentrations of bases. Dreyer and Walker (1914) show that in warm-blooded animals of the same species, but of different weights, dosage of drugs must be calculated in relation to body surface. They explain this on the ground that "the concentration in the plasma of any given substance administered is dependent on the volume of the circulating blood, which is itself proportional to the body surface in any given species of animal." The "basal heat production" of a warm-blooded animal, which may be defined as the heat produced by the animal under consideration when kept at the same temperature as its normal body temperature at rest and starving, is also regarded as proportional to body surface for any given species.

A few experiments were undertaken to see whether any relationship could be traced between the body area or body weight of tadpoles and the effects of acids and bases upon regeneration and carbon dioxide production, which may be regarded as most nearly representing the basal metabolism of the animals.

For the first experiment two tadpoles were taken which had been collected at the same time, and differed only in weight, great care being taken that they should be similar in shape, relative length of tail, etc. After one week of starvation in distilled water, they were weighed and the CO_2

production of each was determined for three successive days (Table 23). While the CO_2 production per gram weight was not the same for the two specimens on any two days, the averages of the three days 3.97 and 3.99 are practically identical.

It was now hoped to determine the areas of the tadpoles by anaesthetizing and making plaster casts of them, which could later be marked off into areas and measured with dividers. This method, however, proved unsatisfactory and was abandoned.

For the next experiment (16) a new method of calculating the area was devised. The average of the length, width and depth of the body was found and with this as the diameter the area of the body was calculated as the area of a sphere. The area of the tail was computed by regarding it as a rectangle from the base to the level at which it begins to taper rapidly to a point, and as a triangle from this level to the tip. These areas were doubled to give the two sides of the tail, and the area of the body, already found, added to give the total area of the tadpole, which is expressed in square millimeters. The area computed by this method is probably considerably less than the actual area of the tadpole, but it was hoped that by selecting animals similar in shape, the ratios of computed area to actual area would be about the same.

Three sets of three tadpoles each were selected, measured, weighed and the CO_2 production determined for six successive days (Table 24). At the end of this time the CO_2 per day per gram of tadpole was 4.65 c.c. for the larger size, 4.66 c.c. for the second size and 5.0 c.c. for the smaller size, showing a slight increase in CO_2 production per unit of weight, with a decrease in size. In order to compare the relative area, weight and CO_2 production of the three tadpoles, all were expressed in terms of per cent of the larger size taken as 100 (Table 24). The weight and CO_2 production vary by differences of +1.13 % and +2.83 % in the second and third sizes respectively, while the

area and CO_2 production vary by differences of -8.8% and -12.6%, suggesting that the normal CO_2 production of tadpoles in distilled water is much more nearly correlated with body weight than with area, as is the case for warm-blooded vertebrates.

The next series (Experiment 17) was carried out in the same manner. Carbon dioxide production was determined over a period of eight days. The large tadpoles used in this experiment were much larger than those used in the preceding, and it was noticed that they frequently exhausted the supply of oxygen in the air in the experimental jars. This was evident because when the jars were opened, although the temperature had remained constant, the lids came off with a "pop", showing that a partial vacuum had been formed. The tadpoles also had a tendency to float at the surface, as they had been observed to do in low oxygen water. For this reason it was rather to be expected that the CO_2 production of the larger size would be proportionately low, and this is found to be the case (Table 25). If now the large tadpoles be taken as standard or 100% in comparing the area, weight and CO_2 production of the three sets, the CO_2 production of the second and third sizes will appear abnormally high. Thus the difference between weight and CO_2 production are +6.8% and +4.2% for the second and third sizes respectively, while the differences in area and CO_2 production are -7.1 and -8.5. Despite the fact that the large tadpoles in which CO_2 production was abnormally low were taken as standard, the CO_2 production still corresponds more nearly to the weight than to the volume. If, on the other hand, the second size of tadpoles be taken as 100%, the relative difference between weight and CO_2 production of the third size is only +1.6%, while the difference between area and CO_2 production is -10.7%, showing again a very close correlation between weight and CO_2 production.

At the close of the eight days in distilled water the CO_2 production

of the same tadpoles was determined for a period of six days in 20 c.c. 0.01 N HCl made up to 400 c.c. - the same volume as had been used in the previous experiments. The CO_2 production decreased in all three sets. Although the absolute decrease was greater in the larger tadpoles, the relative decrease was greater in the smaller ones, the per cent decrease being 38%, 48% and 50% in the large, medium and small sizes respectively.

If the decrease in CO_2 production be compared to weight and area, using the large size as 100% (Table 25), the decrease in CO_2 production is seen to be more nearly correlated with area than with volume. This experiment, while suggestive, is not conclusive, however, as there were no distilled water controls and as the CO_2 production in distilled water showed a continuous decrease even before the acid was added.

In the next series (Experiment 18) the error due to the larger tadpoles exhausting the air in their jar was avoided by putting the same weight rather than the same number of tadpoles in each jar. The jars were divided into four sets,each containing three sizes of tadpoles numbered A, B, and C (Table 26). The large tadpoles (A) were hardly comparable with the other two sizes, as all had hind legs in which the second joint had already developed. Nos. 1A and 3A especially, were different, the former because it had a very large compact body and short, stubby tail, the latter because the body was rather long and the legs were much farther developed than in any of the others. The individual tadpoles were weighed and measured and the areas computed as in the above experiments. However, only the summaries are given here (Table 26). After the CO_2 production had been determined for six successive days in distilled water, set 1 was kept as a control, while sets 2, 3 and 4, were put in 20 c.c. of KOH, $Ca(OH)_2$ and H_2SO_4 respectively, made up to 400 c.c. The CO_2 production in these solutions was determined for four days. An examination of the summary (Table 26) shows that while the CO_2 production per gram varies from

5.65 to 6.45 c.c. per day, even if we exclude the two individuals (1A and 3A) already mentioned as aberrant, that this variation bears no apparent relation to size and that the average CO_2 production is about the same for all sizes, thus for size B it is 6.04 and for size C 5.96.

Due to the early death of some of the tadpoles and abnormal behavior of others during the experiment, no attempt is made to correlate the increased CO_2 in bases or the decreased CO_2 in H_2SO_4 with either weight or area. The experiment does show, however, that the per cent of increase of CO_2 in bases and the per cent of decrease of CO_2 in acids both increase as the size of the tadpoles decrease and, as the relative area of the tadpoles increases as the size decreases, this fact may be regarded as somewhat suggestive.

The surviving tadpoles of the above experiment were operated upon and allowed to undergo regeneration in the same concentrations as the CO_2 determinations had been made for. The results (Table 27) show that the concentrations used had no effect upon regeneration in size A. That size B was affected only in the case of H_2SO_4, in which regeneration was noticeably retarded, while in those of size C which survived to regenerate, the regeneration was markedly depressed.

For the final series on the affect of size on regeneration (Experiment 18) 0.01 N HCl was used in concentrations of 30, 40, 50 and 60 c.c. per liter. The experiments were carried out in 10-inch crystallizing dishes, each initially containing ten tadpoles of three different sizes. Two tadpoles in the first control and one in 40 c.c. HCl jumped out during the first few days. All in 60 c.c. died without regeneration within ten days. An examination of the data (Table 28) shows that under normal conditions the average per cent of regeneration increases as the size of the tadpoles decreases. The per cent of regeneration as compared to the controls in various concentrations of HCl is practically the same for all. (The high per cent of regeneration, as compared to the controls shown by the tadpoles of medium size, is due to the

abnormally low regeneration of the tadpoles of that size in the first control). A comparison of this experiment with Experiment 18 (Table 27) seems to suggest that the depression of regeneration due to acids is practically the same for all sizes of tadpoles until a concentration is reached which is soon fatal to the smaller ones. In such a concentration, those small ones which survive to regenerate, show a strong depression of regeneration (Table 27). This would indicate that the threshold of toxicity of the lower animals is lower in relation to the threshold of depression of metabolism than in the larger animals or, in other words, that the effect of the environment upon the regenerating tissue is comparatively independent of its effect upon the tadpole as a whole. While the data here presented are both too meager and too inconclusive to warrant any conclusions, these experiments and observations upon animals of various sizes in the previous experiments have led the author to regard this as a promising method of attack for future investigations upon the relation between new tissue and the organism as a whole.

Regeneration in Low Oxygen.

The method of obtaining a gradient of oxygen has already been described. For the first series of experiments three tadpoles of different sizes were placed in each of the seven experimental jars. During this experiment the water was strongly basic to Phenolpthalein though acid to Thymolpthalein as no attempt was made to acidulate the water. The data for the larger tadpoles, 70 to 77 mm., are given in Table 29. The tadpole in the first jar (oxygen free water) died without regeneration. The curve of growth (Fig. 34) of No. 3-in 1.6 c.c. O_2 per liter - lies above those of 5 and 6 - in 3.03 and 3.8 c.c. O_2 per liter respectively. This is doubtless accounted for by the fact that Nos. 2 and 3 remained most of the time at the surface of the water, while Nos. 4, 5, 6 and 7 remained at the bottom, coming to the top only occasionally.

When the fact that the water in which Nos. 2 and 3 actually remained contained considerably more oxygen than was shown by the titrations is taken into consideration, these curves (Fig. 34) show clearly a relation between regeneration and the oxygen content of the water.

The same thing is true of the medium-sized tadpoles (Table 30, Fig. 35). Of these Nos. 1 and 3 remained at the top and underwent regeneration, No. 2, which did not show this response, died without regeneration, Nos. 4, 6 and 7, which remained at the bottom, undergo progressively better regeneration. The data for the small-sized tadpoles, 34 to 37 mm., are given at the bottom of Table 30. These results are quite typical of what occurred each time an attempt was made to use small tadpoles in oxygen experiments. Nos. 1, 2 and 3 remained at the top of the water. Nos. 2 and 3 lived twelve days and underwent slight regeneration. Nos. 4, 5, 6 and 7 stayed at the bottom, and of these only No. 7 survived to undergo any regeneration, but its regeneration was apparently normal.

Here again, as was shown for acids and bases, we find the susceptibility of the tadpole as a whole as compared to the susceptibility of the regenerating part higher in smaller than in larger tadpoles.

The next series of experiments was carried on in water kept basic to Rosalic acid and from acid to faintly basic to Phenolpthalein. The temperature was also corrected by the introduction of the air coil which warmed the water to air temperature before it entered the experiment jars. All of the tadpoles chosen were large and showed rudimentary hind legs, as it was found that tadpoles at this stage remained at the bottom of the jars better than those in earlier stages. The results (Table 31, Fig. 36) show an increase in regeneration with an increase in O_2 content of the water with abrupt rises between 0 and 0.7 c.c. per liter, and again between 3.68 and 4.1 c.c. per liter.

In the following experiment still older tadpoles were used.

These had a total length of 75 to 85 cc. and the hind legs were two-jointed and digited, and stood out from the body so as to be visible from the dorsal aspect. The results (Table 33, Figure 37) again show a close correspondence between the amount of oxygen and the rate of regeneration. Figure 38 is a curve representing the length of time necessary to regenerate 20% of the amount removed in the increasing concentrations of oxygen. As has been mentioned before, this curve is similar to the curves of Krogh (1914a) for the development of frog eggs at increasing temperatures.

The final experiment of the oxygen series owes its value to an accident. The tadpoles of this series were 71 to 76 mm. long and had rudimentary hind legs. No. 3 died without regeneration. Of the other five, Nos. 1 and 2, in 0.2 and 1.5 c.c. O_2 per liter respectively showed marked retardation of regeneration when measured on the twenty-first day (Table 33, Fig. 39). During the twenty-second night, the flow of water through the jars stopped, due to clogging of the boiling apparatus by sediment from the water,and before this could be remedied the water in the jars had become well aerated. During the following night the water again ceased to flow through the jars and again the water in which the tadpoles were became aerated. Since the tadpoles had been for two days in aerated water, the original purpose of the experiment had to be abandoned. An aerating jar with a rapid stream of air passing through it was placed just after the mixing bottle so that the water entered the first jar aerated. Measurements made on the twenty-sixth day of the experiment showed that the tadpole in the first jar (0.2 c.c. O_2 per liter),which had regenerated only 2.8% during the twenty-two days preceding the aeration of the water, had regenerated 19.8% in the four days following the change. The tadpoles already in higher amounts of O_2, of course, showed less difference. This experiment is valuable as demonstrating that the decrease in regeneration

in the lower amounts of oxygen is due to the lack of oxygen and not to any slight rise in temperature of the water in the successive jars, or to a decrease in alkalinity of the water as it took up carbon dioxide produced by the successive tadpoles.

It has long been known that low temperatures, if not so low as to produce detrimental effects, may retard or completely check the development of certain animals for a considerable period of time, the animal still retaining the power to complete normal development. The following experiment was planned to test whether this might be true for regeneration. Three sets of four tadpoles each were selected. The first set was kept at room temperature - 19 to 21 degrees centigrade - the second was kept in a water bath at 14 degrees, and the third was kept in a refrigeration tank at 0 to 4 degrees. The first set underwent regeneration rapidly, the rate of the second set was considerably retarded, the third set underwent no regeneration whatever (Table 34 and Fig.40). When the first set had completed regeneration, two from the third set were transferred to room temperature. The day in which they were transferred and several succeeding days were cool, so the temperature of the water stood at 18 to 19 degrees. For this reason the regeneration of these tadpoles was slightly slower than that of the controls, although the growth curves are very similar. The two tadpoles left at the lower temperature died without regeneration at the end of forty-seven days.

In comparing the effects upon metabolism of hydrogen ion concentration, low oxygen and low temperature, it is of interest to note that alike they cause decrease and both the rate of regeneration and the total amount regenerated. Were the effect of these agents due wholly to their action in retarding the metabolism and consequent division and growth of the cells of the regenerating parts, the result would not be a decrease in the ultimate amount regenerated, but rather an increase in the length of time necessary to complete

regeneration. This, however, is not the case for in acids and bases the tadpoles in the higher and more detrimental concentrations cease regenerating at a time as early or earlier than the controls. In low oxygen regeneration ceases at practically the same time in all concentrations, while in low temperatures, although the period of growth is longer at the lower temperatures than at the higher temperatures, the difference is not sufficient to compensate the difference in rate of regeneration, so the ultimate amount regenerated is still less at the lower than at the higher temperatures.

The only tenable explanation seems to be that the phenomena of growth and of differentiation in the regenerating parts are differently affected by the environmental agents studied, that is, that the optima for these phenomena are different so that a hydrogen ion concentration, an oxygen content or a temperature of the surrounding medium which retards one process may have no effect upon the other. Thus a hydrogen ion concentration or deficiency in oxygen which causes a marked retardation to regeneration may have no effect upon differentiation, while a temperature which retards regeneration greatly may have only a slightly retarding effect upon differentiation.

That the three environmental factors studied may differ this way in regard to their relative effects upon regeneration and differentiation seems the more probable since experiments show that they do differ in their relative effects upon regeneration and survival or length of life. Thus a concentration of acids or bases or a deficiency of oxygen sufficient to completely check regeneration is rapidly fatal, while a tadpole may live forty-eight days at a temperature which completely prevents regeneration.

SUMMARY

A study has been made of the effects of various conditions of hydrogen ion concentration, deficiency of oxygen and low temperature upon regeneration and metabolism in tadpoles of Rana climata.

These studies show that the optimum hydrogen ion concentration for regeneration is neutrality or very near neutrality. As the hydrogen ion concentration varies from neutrality in the direction of either acidity or bacisity, both the rate of regeneration and the total amount regenerated decrease, at first gradually and then very rapidly. These effects are due to the hydrogen and hydroxyl ions.

The relative effect upon regeneration of any given hydrogen ion concentration is practically the same for all stages of regeneration.

In water of low oxygen content, both the rate of regeneration and the total amount regenerated are proportional to the oxygen present. Both the rate of regeneration and the amount regenerated decrease with a decrease of temperature.

Tadpoles, whose regeneration has been completely checked by a decrease in temperature, retain the capacity to undergo a large degree of regeneration to or beyond a period at which control tadpoles have completed regeneration. Tadpoles, whose regeneration has been checked by insufficient oxygen, show this capacity to a less degree, while those whose regeneration has been checked by acids or bases show it scarcely at all. It is suggested that this may be due to a difference in the relative effects of the environmental factors studied upon the phenomena of regeneration and differentiation.

Carbon dioxide production of tadpoles in neutral water is proportional to their weight.

Carbon dioxide production is decreased by acids and increased by bases. A high rate of carbon dioxide production, when produced by a basic

medium, is not correlated with a high rate of regeneration. Both the relative decrease of carbon dioxide production in acids and the relative increase of carbon dioxide production in bases increase as the size of the tadpoles decrease. This is suggestive of a correlation between the effect of acids and bases and the area of the tadpole. The relative decrease in regeneration of tadpoles due to acids and bases appears to be independent of size, excepting in concentrations which are soon fatal to the smaller ones.

The probability is suggested that hydrogen ion concentration, insufficient oxygen, low temperatures and toxic substances affect development, regeneration, oxygen metabolism, and duration of life, according to the same laws, although the relative effects of any given environmental factor upon the various functions of the organism differ, and although the relative effects of the same environmental factor may differ in animals of different size.

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TABLE 1 EXPERIMENT 1

Regeneration in Bases - KOH

cc. 0.9N KOH in 200 cc. H_2O	Length in mm.	Length removed in mm.	Length Regenerated in mm.					
date - begun		10/25/17	10/30	11/1	11/2	11/3	11/5	11/7
0	55.4	14.3	1.2	2.1	2.2	2.8	3.4	4.2
%regen.			8.4	14.7	15.4	19.5	23.8	29.4
1/4	53	12.0	1.3	2.0	2.5	2.9	3.1	3.7
%regen.			10.8	16.8	20.8	24.	26.	31.
1/2	54	12.5	1.1	2.1	2.5	2.9	3.3	4.0
%regen.			8.8	16.8	20.	23.	26.4	32.
2'	55.7	12.9	1.1	2.1	2.2	2.5	3.2	4.2
%regenerated			8.5	16.3	17.	19.4	25.	32.5
5	57	13.3	1.0	2.1	2.6	3.0	3.7	4.4
%regen.			7.5	15.8	19.5	22.6	28.	33.
10	50	12.0	0.8	1.9	2.0	2.5	3.4	4.0
%regen.			6.6	15.8	16.8	29.4	28.3	33.3
15	59	13.8	0.8	1.2	1.3	2.0	2.8	3.3
%regen.			5.8	8.7	9.4	14.5	20.	24.
20	52	12.9	0.2	0.8	1.1	1.7	2.0	2.6
%regen.			1.5	6.2	8.5	13.2	15.5	20.
25	55.5	12.1	died without regeneration					

continued below

Date - -	11/9	11/12	11/14	11/18	11/20	11/22	11/24
0	4.6	5.1	5.6	5.8	6.4	6.2	6.2
%reg.	32.	36.	29.	40.5	45.	43.5	43.5
1/4	4.7	5.0	5.1	5.4	5.8	5.8	5.8
%reg.	39.	41.	42.5	45.	47.5	47.5	47.5
1/2	4.3	4.8	4.8	5.0	5.5	5.5	5.2
%reg.	34.4	38.	38.	40.	44.	44.	42.
2	4.3	5.0	5.1	5.3	5.3	5.4	5.3
%reg.	33.3	39.	29.5	41.	41.	41.	41.
5	4.6	4.6	4.6	4.7	4.8	5.1	5.1
%reg.	34.6	34.6	34.6	35.3	36.	38.4	38.4
10	4.4	4.0	4.0	4.0	4.0	4.0	4.0
%reg.	36.6	39.	39.				
15	3.5	3.6	3.6	3.8	dead		
%reg.	25.	26.	26.	27.5			
20	3.1	3.3	3.3	dead			
%reg.	24.	25.6	25.6				

TABLE 2 EXPERIMENT 1

Regeneration in Basas------ KOH

cc .01 N KOH in 200 cc.H_2O	Length in mm=	Length removed in mm.	Length Regenerated in mm.						
date	- begun	10/25/17 -	10/30	11/1	11/2	11/3	11/5	11/7	11/9
0	23.7	5.3	1.2	2.1	2.3	2.6	2.9	3.0	3.2
%regenerated			23.	40.	44.	49.	55.	56.5	60.
½	26.	6.1	1.3	1.9	2,0	2.1	2.6	2.6	2.9
%regenerated			21.	31.	33.	34.	42.6	42.6	48.
1	27	6.1	1.4	2 .0	2.4	2.7	3.0	3.1	3.1
%regenerated			23.	33.	39.	44.	49.	51.	51.
2	26	5.9	1.1	2.2	2.1	2.2	2.6	2.6	2.7
%regenerated			20.4	37.	37.	37.	44.	44.	46.
5	28.5	5.2	1.2	2,0	2.0	2.2	2.5	2.7	2.7
%regenerated			23.	38.5	38.5	42.	48.	52.	52.
10	26.5	5.5	1.0	1.5	1.5	2.0	2.0	2.1	2.2
%regenerated			18.2	27.3	27.3	36.4	36.4	38.	40.
15	26.	4.8	0.6	0.9	0.9	1.1	1.3	1.6	1.8
%regenerated			12.5	18.7	18.7	23.	27.	33.3	37.5
20	28.	6.0	died without regeneration.						

continued from above

KOH	date	11/12	11/14	11/17	11/20	11/22
0		3.2	3.3	3.3	3.2	3.2
%regen.		60.	62.	62.	60.	60.
½		2.9	2.9	3.0	3.0	3.0
%regen.		48.	48.	49.	49.	49.
1		3.1	3.1	3.2	3.2	3.2
%regen.		51.	51.	52.	52.	52.
2		2.7	2.7	3.0	3.0	dead
%regen.		46.	46.	51.	51.	
5		2.7	2.7	2.7	dead	
%regen.		52.	52.	52.		
10		2.2	dead			
		40.				
15		1.0	dead			
%regen.		20.8				

TABLE 3 EXPERIMENT 2

Regeneration in Bases - $Ca(OH)_2$

No.	$Ca(OH)_2$ in 200 cc. H_2O	Length in mm.	Length removed in mm.	Length Regenerated in mm.					
	date -	begun	10/30/17	11/4	11/7	11/9	11/12	11/14	11/17
1	0	50	11.8	1.0	3.0	3.2	4.0	4.7	5.0
		%		8.5	25.4	27.	34.	41.	42.
1'	0	45	11.0	1.0	3.1	3.4	4.5	4.6	4.6
		%		9.0	28.	31.	41.	42.	42.
2	¼	47	12.0	1.1	3.0	3.8	4.5	4.5	4.7
		%		9.0	25.	31.3	37.2	37.2	39.1
3	½	46	13.1	1.1	3.0	4.1	5.3	5.8	6.1
		%		8.4	22.9	30.9	41.	44.	46.5
4	1	45	11.1	0.9	2.8	3.4	4.2	4.2	4.3
		%		8.1	25.2	30.6	38.	38.	38.
5	2	46	12.1	1.0	3.1	4.1	5.0	5.1	5.1
		%		8.3	25.8	34.	41.6	42.5	42,5
6	5	44	11.3	0.9	3.0	3.8	4.6	4.8	4.8
		%		8.0	26.6	33.8	40.7	42.5	42.5
7	7.5	49	11.0	0.6	2.2	3.2	4.0	4.0	4.0
		%		5.5	20.	29.	36.	36.	36.
8	10	45	12.0	0.4	2.5	3.1	4.5	4.4	4.3
		%		3.3	20.8	25.8	37.4	37.	37.
9	15	47.	12.0	0.2	2.2	3.0	3.1	3.1	3.2
		%		1.6	18.4	21.	26.	26.	26.8
10	17.5	48	11.2	- -	2.0	2.4	2.8	2.8	dead
		%			18.	21.4	25.	25.	
11	20	48	10.0	died without regeneration.					

continued below

No.	$Ca(OH)_2$	date	11/19	11/22	11/27	11/30
1	0		5.4	5.5	5.5	5.5
		%	46.	51.	51.	51.
1'	0		4.7	5.0	5.0	5.0
		%	43.	45.	45.	45.
2	¼		5.0	5.0	5.0	5.0
		%	41.6	41.6	41.6	41.6
3	½		6.5	6.5	6.5	6.5
		%	49.	49.	49.	49.
4	1		4.4	4.8	5.0	5.0
		%	39.6	43.	45.	45.
5	2		5.3	5.7	5.7	5.7
		%	44.	47.5	47.5	47.5
6	5		5.0	5.0	dead	
		%	44.	44.		
7	7.5		4.8	4.8	4.9	5.0
		%	43.	43.5	44.5	45.4
8	10		4.3	4.4	5.0	5.0
		%	37.	37.	41.	41.
9	15		3.1	3.1	3.3	3.8
		%	26.	26.	27.5	31.6
10						

TABLE 4 EXPERIMENT 2

Regeneration in Bases - $Ca(OH)_2$.

No.	$Ca(OH)_2$ in 200 cc.H_2O	Length in mm.	Length removed in mm.	Length Regenerated in mm.						
	date -	begun	10/30/17	11/4	11/7	11/9	11/12	11/14	11/17	
1	0	31	7.0	1.1	2.3	2.4	2,8	2.8	3.0	
		%		16.	33.	34.	40.	40.	43.	
1'	0	31	6.9	1.2	2.2	2.6	2.6	2.6	2.8	
		%		18.	32.	37.	37.	38.	40.	
2	¼	32	7.0	1.2	2.2	2.7	3.0	3.3	3.3	
		%		17.	31.2	38.4	43.	47.	47.	
3	½	32	6.3	1.0	2.3	2,5	3.0	3,0	3.0	
		%		16.	36.3	40.	47.6	47.6	47.6	
4	1	31	6.6	1.0	1.9	2.0	2.4	2.5	2.6	
		%		15.	29.	30.3	36.4	38.	39.4	
5	2	31	7.1	1.2	2.1	2.6	3.0	3.3	3.3	
		%		17.	29.6	36.6	42.	46.5	46.5	
6	5	30.	7.0	1.1	2.4	3.0	3.2	3.2	3.5	
		%		15.4	34.3	43.	46.	46.	50.	
7	7.5	32	6.8	1.0	2.0	2.3	2.6	3.0	2.8	dead
		%		14.7	29.4	34.	38.	44.	41.2	
8	10	28.5	6.0	1.0	1.9	2.1	2.2	dead		
		%		16.6	31.7	35.	37.			
9	15	30	6.3	0.9	1.9	2.0	2.2	2.2	dead	
		%		14.3	30.	32.	35.	35.		
10	17.5	31	7.1	0.7	1.1	1.6	dead			
		%		10.	15.7	22.6				
11	20	34	6.5	0.3	dead					
		%		4.6						

continued below

No.	$Ca(OH)_2$	date	11/19	11/22	11/27
1	0		3.0	3.0	3.0
		%	43.	43.	43.
1'	0		2.9	2.9	2.9
		%	42.	42.	42.
2	¼		3.3	3.3	3.3
		%	47.	47.	47.
3	½		3.0	3.0	3.0
		%	47.6	47.6	47.6
4	1		2.7	2.7	dead
		%	41.	41.	
5	2		3.3	3.3	3.3
		%	46.5	46.5	46.5
6	5		3.5	3.5	3.5
		%	50.	50.	50.

TABLE 5 EXPERIMENT 3

Regeneration in Bases - Na_2CO_3

.01 mol. Na_2CO_3 in 200 cc.H_2O	Tad. no.	Length in mm.	Length removed in mm.	Length Regenerated in mm.										Approximate PH	
Begun 3/27/18				4/2	4/4	4/6	4/8	4/10	4/14	4/18	4/22	4/24	4/28	initial	after 48 hrs.
0	1	49	11.8	2.2	3.4	5.0	6.0	7.0	7.5	5.3	5.9	5.9	5.9	7	6.8
	2	50	13.2	2.5	4.0	3.8	4.4	4.9	5.0	8.0	8.4	8.5	8.5		
	3	49	11.8	3.2	3,1	3.7	4.7	5.0	5.6	6.0	6.0	6.0	6.0		
	4	44	12.0	2.0	3.2	4.2	5.0	5.2	5.6	5.6	6.0	6.0	6.0		
	total		48.8	8.9	13.7	16.7	20.1	22.1	23.7	24.9	26.3	26.4	26.4		
	%			18.2	28.	34.	41.	45.	48.5	51.	54.	54.	54.		
15	1	47	12.1	1.6	2.8	3.8	5.0	4.9	5.1	5.5	5.7	5.7	5.7	10+	7.4
	2	49	12.0	1.5	3.2	3.5	4.4	5.0	5.5	5.7	6.0	6.1	6.1		
	3	45	11.8	2.0	3.1	4.0	4.5	5.0	5.6	5.5	5.0	5.0	5.0		
	4	44	13.0	2.2	3.2	3.5	4.2	4.7	5.4	5.6	6.0	6.0	5.6		
	Total		48.9	7.3	12.3	14.8	18.1	19.6	21.6	22.3	22.7	22.8	22.4		
	%			14.9	25.2	30.2	37.	40.	44.	46.	46.5	46.7	46.		
30	1	48	13.5	1.3	1.7	2.2	2.3	4,2	3.9	3.3	4.0	4.0	4.0		7.8
	2	50	12.5	1.7	2.1	3.0	3.7	2.9	3.3	4.8	5.0	5.1	5.1		
	3	45	11.3	1.7	2.7	3.4	3.1	3.1	5.0	3.9	3.9	3.9	4.0		
	4	43	11.2	1.8	2.8	3.2	3.7	3.8	4.7	5.0	5.0	5.2	3.8		
	total		48.5	6.5	9.3	11.8	12,8	14.0	16.9	17.0	17.9	18.2	16.9		
	%			13.4	19.2	24.4	26.4	29.	35.	35.	37.	37.5	34.		
45	1	52	12.1	1.1	1.1	1.7	2.0	2.3	2.3	2.1	dead				8
	2	48	13.0	1.0	1.1	1.8	1.8	1.8	1.8	1.8	dead				
	3	47	11.9	0.6	1.8	2.4	dead								
	4	40	11.0	1.0	1.8	1.8	dead								
	total		48.	3.7	5.7	7.7	3.8	4.1	4.1	3.9					
	%			7.7	12.6	16.	15.1	16.3	16.3	15.5					
60															
	died without regeneration.														
75															
0	1	52	11.5	2.6	3.1	3.5	4.3	4.3	5.2	5.9	6.0	6.1	6.6	7	6.8
	2	48	12.9	3.2	3.3	4.0	4.0	5.1	4.3	5.4	6.5	6.5	6.0		
	3	43	11.6	2.2	3.2	3.9	4.1	4.8	5.1	5.1	5.5	6.0	6.4		
	4	44	12.2	2.4	3.2	4.0	4.5	5.2	5.7	6.1	6.8	6.7	5.6		
	total		48.2	9.4	12.8	15.4	16.9	19.4	20.3	22.5	24.8	25.3	24.0		
	%			19.5	26.6	32.	35.	40.3	42.	47.5	51.5	52.5	50.		

TABLE 5a EXPERIMENT 3

Regeneration in Bases NaHCO$_3$

.01 mol. NaHCO$_3$ in 200 cc.H$_2$O	Tad. no.	Length in mm.	Length removed in mm.	Length Regenerated in mm.										Approximate PH	
	Begun 3/27/18			4/2	4/4	4/6	4/8	4/10	4/14	4/18	4/22	4/24	4/28	initial	after 48 hrs.
0	controls the same as for Na$_2$CO$_3$														
15	1	48	12.6	2.0	3.2	4.2	4.8	4.9	5.2	6.0	6,0	6.0	6.0	7.7	7.2
	2	49	12.0	2.3	4.0	4.1	5.7	6.4	6.9	8.0	8.0	8.0	8.0		
	3	48	12.0	1.8	2.6	3.4	4.4	5.0	5.8	5.7	6.0	6.1	6.1		
	4	49	11.8	1.7	3.0	3.3	3.6	2.8	3.2	missing					
	total		48.4	7.8	12.8	15.0	18.5	19.1	21.1	19.7	20.	20.1	20.1		
	%			16.1	26.5	31.	38.2	39.5	43.5	51.	54.5	55.	55.		
30	1	48	12.5	2.0	3.0	3.7	4.1	4.2	4.9	4.9	5.0	5.0	5.0	7.9	7.5
	2	52	12.0	2.1	3.1	3.6	4.1	4.5	4.2	4.9	5.1	5.1	5.1		
	3	45	12.0	2.1	3.1	3.6	3.9	4.0	4.8	4.5	5.1	5.1	5.0		
	4	45	11.8	1.8	2.8	3.2	4.0	4.2	4.5	4.5	4.5	4.5	4.5		
	total		48,3	8.0	12.0	14.1	16.1	16.9	18.4	18.8	19.7	19.7	19.6		
	%			16.5	25.	29.	33.4	35.	38	39.	40.7	40.7	40.6		
60	1	52	12.6	2.0	3.3	3.6	4.1	4.6	5.3	5.6	5.5	5.5	5.5	8.3	7.8
	2	47	11.2	2.1	2.7	3.8	4.3	5.0	4.6	5.0	5.0	5.3	5.3		
	3	43	11.4	1.9	2.8	3.4	4.0	4.2	4.8	4.9	5.0	5.0	5.0		
	4	51	13.0	1.9	2.6	3.4	3.8	4.0	missing						
	total		48.2	7.9	11.4	14.2	16.2	17.8	14.7	15.5	15.5	15.8	15.8		
	%			16.4	23.6	29.4	33.6	37.	42.	44.	44.	45.	45.		
45	1	58	13.6	2.0	3.4	4.1	4.1	4.8	5.8	5.7	6.2	6.3	5.7	8.1	7.7
	2	52	12.2	2.1	3.0	3.9	4.8	5.3	6.0	6.2	5.7	5.7	6.3		
	3	46	12.5	2.1	2.5	3.7	4.2	4.6	4.8	5.2	5.2	5.2	5.2		
	4	41	10.5	1.6	2.9	3.1	4.0	4.0	5.0	5,0	5,0	5,0	5.0		
	tOtal		48.8	7,8	11.8	14.8	17.1	18.7	21.6	22.1	22.1	22.2	22.2		
	%			16.	24.2	30.4	35.	38.	44.	45.	45.	45.5	45.5		
75	1	55	13,1	1.9	2.3	3.3	3.3	3.9	4.2	6.0	6.0	6.0	6.0	8.5	7.9
	2	50	13.0	1.8	3.1	3.3	4.0	4.6	5.2	4.9	5.0	5.2	5.2		
	3	45	11.3	2.1	3.2	3.5	4.1	4.6	4.9	5.1	5.1	5.1	5.1		
	4	43	11.8	2.0	3,0	3,5	4.1	4.1	5.1	missing					
	total		49.2	7.9	11.6	13.6	15.5	17.3	19.4	16.0	16.1	16.3	16.3		
	%			16.	23.6	27.6	31.5	35.	39.5	43.	43.	43.5	43.5		
90	1	54	12.7	2.1	3.2	3.3	4.0b	5.0	5.6	5.8	5.6	5.8	5.8	8.6	8
	2	49	12.3	2.0	2.8	4.1	4.7	5.0	5.7	6.1	6.1	6.1	6.0		
	3	47	13.0	1.8	3.1	3.3	3.9	4.5	4.8	5.5	5.5	5.3	5.3		
	4	44	11.3	1.7	2.8	3.1	3.8	3.8	4.2	4.9	4.9	4.9	4.9		
	total		49.3	7.6	11.9	13.8	16.4	18.3	2013	22.3	22.1	22.1	22.0		
	%			15.4	24.	28.	33.	37.	41.	45.	45.	45.	45.		
120	1	51	12.0	1.9	2.5	3.0	3.1	3.2	3.3	4.1	3.3	3.3	3.3	8.7	8.1
	2	51	11.9	2.0	3.0	3.1	3.6	3.8	4.0	3.0	4.1	4.2	4.2		
	3	45.8	11.8	2.0	2.6	3.0	3.1	3.2	3.5	3.2	3.5	3.5	3.5		
	4	45	12.0	2.0	2.3	2.7	2.8	3.0	3.0	3.3	3.1	3.2	3.2		
	total		47.7	7.9	10.4	11.8	12.6	13.2	13.8	13.6	14.0	14.2	14.2		
	%			16.5	22.	25.	26.4	27.6	29.	28.5	29.	29.7	29.7		

TABLE 6 EXPERIMENT 3

Regeneration in Bases - Na_2CO_3 + $NaHCO_3$

Na_2CO_3 in 200 cc.H_2O	$NaHCO_3$ in 200 cc.H_2O	Tad. no.	Length in mm.	Length removed in mm.	Length Regenerated in mm.										Approximate PH	
		begun	3/27/18		4/2	4/4	4/6	4/8	4/10	4/14	4/18	4/22	4/24	4/28	initial	after 48 hrs.
0	0	1	50	12,5	2.0	3.1	3.6	4.1	4.6	5.3	5.8	6.0	6.1	6.1	7	6.8
		2	54	12.5	2.1	3.0	3.8	dead								
		3	43	11.6	2.1	3.8	4.2	5.0	5.3	5.2	6.0	6.5	6.1	6.1		
		4	47	12.1	2.4	3.8	4.1	5.0	5.1	5.9	6.0	6.0	6.5	6.5		
		total		48.7	8.6	13.7	15.7	14.1	15.0	16.4	17.8	18.5	18.7	18.7		
		%			18.	28.	33.	38.	40.5	44.	48.	50.	50.	50.		
15	45	1	49	11.8	1.8	2.5	3.0	3.6	3.9	3.9	4.1	4.1	4.1	4.1	9.7	7.5
		2	47	12.0	2.0	2.9	3.3	3.9	4.0	5.0	5.5	4.9	4.9	4.9		
		3	44	11.6	2.1	2.8	3.1	3.8	3.8	4.5	4,8	4.4	4.4	4.4		
		4	45	11.0	2.0	3.0	3.3	3.7	4.2	4.0	4.3	5.8	5.8	5.7		
		total		46.4	7.9	11.2	12.7	15.0	15.9	17.4	18.7	19.2	19.2	1911		
		%			17.	24.2	27.5	32.4	34.3	37.5	41.	41.5	41.5	41.5		
30	30	1	51	13.0	1.4	2.2	2.7	3.0	3.1	3.5	3.6	3.6	3.6	3.6	10+	7.8
		2	53	13.5	1.1	2.0	2.7	2.9	2.8	2.8	3.2	3.5	3.7	3.7		
		3	44	11.4	1.0	2.3	2.8	3.0	3.0	3.1	3.5	3.8	4.0	4.0		
		4	50	11.8	1.6	2.1	2.2	2.3	2.5	2.8	3.1	3.0	3.0	dead		
		total		49.7	5.1	8.6	10.4	11.2	11.4	12.2	13.4	13.9	14.3	11.3		
		%			10.2	17.5	21.	22.5	23.	24.5	27.	28.	29.7	30.		
45	15	1	47	11.8	0.8	1.1	1.6	1.4	1.7 dead							
		2	53	11.6	1.1	1.2	1.7	2.1	dead							
		3	42	11.5	1.0	1.2	1.3	1.8	2.0 dead							
		4	45	12.8	1.0	2.0	2.1	2.2	dead							
		total		47.7	3.9	5.5	6.7	7.5	3,7							
		%			8.2	11.5	14.	15.7	15.7							
50	25	1	52	12.8	0.7	dead										
		2	49	11.5	0.6	dead										
		3 3	46	12.0	-	dead										
		4	46	11.8	-	0.9	1.1	1.3	dead							
		total		48.1	1.3	0.9	1.1	1.3								
		%			5.2	7.6	9.34	11.0								
25	50	1	52	13.0	1.7	2.1	2.4	2.9	2.9	3.1	3.7	3.7	3.7	3.7		8
		2	51	11.6	1.8	2.5	2.8	3.7	3.7	3.7	3.3	3.4	3.3	dead		
		3	44	12.8	1.5	2.2	3.1	3.0	3.0	3.2	3.8	3.7	3.8	3.8		
		4	46	11.7	1.3	2.3	2.4	2.9	3.0	3.0	3.3	3.4	3.4	3.4		
		total		48.1	6.3	9.1	10.7	12.5	12.6	13.0	14.1	14.2	14.2	10.9		
		%			13.1	19.	22.2	26.	26.2	27.	29.3	29.5	29.5	30.		

TABLE 7 EXPERIMENT 4

Carbon dioxid Production in Bases - KOH.

No.	ccKOH in 400 cc. H_2O	CO_2 as cc. 0.01N H_2CO_3								
	date - -	1/17	1/18	1/20	1/21	1/22	1/23	1/24	1/25	1/26
1	0	36.2	47.7	44.6	41.0	48.9	33.9	36.3	58.5	44.5
2	15	37.5	41.5	52.9	46.8	43.7	44.7	44.9	48.0	43.0
3	25	44.0	47.5	45.5	49.2	48.6	50.6	46.0	52.0	61.0
4	30	49.7	55.0	dead						
5	35	48.6	dead							
6	40	51.3	dead							

No.	KOH	date 1/27	1/28	1/29	1/30	1/31 2/1	2/2 2/3	2/4	2/5	2/6
1	0	31.3	42.8	37.0	32.0	43.6	38.2	27.6	27.4	27.8
2	15	31.7	51.8	43.0	37.5	53.5	41.3	32.7	28.1	30.5
3	25	43.4	70.0	48.0	48.7	59.8	57.0	41.0	41.6	43.3

No.	KOH	date 2/8	2/9	2/10	2/11	wt.in gms.	days	total CO_2	CO_2 per day
1	0	22.5	27.2	29.6	20.8	6.9	24	799.3	33.3
2	15	27.4	29.5	32.5	29.0	6.9	24	863.9	36.0
3	25	37.0	38.0	39.7	37,5	7.2	24	1038.4	43.25

No.	KOH	CO_2 per day gram.
1	0	4.83
2	15	5.22
3	25	6.01

TABLE 8 EXPERIMENT 4

Regeneration in Bases - KOH.

No.	cc.KOH in 400 cc.H_2O	Tad. no.	Length in mm.	Length removed in mm.	Length Regenerated in mm.					
	date - begun 1/17/18				1/28	2/2	2/7	2/12	2/17	2/22
1	0	1	71	13	4.0	6.7	6.7	7.0	7.4	7.4
		2	67	12	3.5	5.2	6.2	6.6	6.8	6.8
		3	64	11.1	died					
		* 4	55	10	1.2	2.0	2.1	3.2	3.2	3.2
		Total		25	7 .5	11.9	12.9	13.6	14.2	14.2
		%			30.0	47.6	51.6	54.4	54.9	54.9
2	15	1	69	13	died					
		2	65	12.6	3.3	5.0	6.2	6.2	6.2	6.2
		3	63	11.8	3.6	5.3	7.0	7.0	7.0	7.0
		4	58	10	2.5	3.8	3.8	3.8	3.8	3.8
		Total		34 .4	9.4	14.1	17.0	17.0	17.0	17.0
		%			27.3	41.	50.	50.	50.	50.
3	25	1	74	13	1.0	2,5	4.0	4.2	3.9	3.9
		2	70	13,5	2.1	3.3	4.1	5.0	dead	
		3	62	12.	2.0	3.0	3.0	3.8	dead	
		4	57	10	2.7	3.7	5.2	5.3	dead	
		Total		48 .5	7.8	12.5	16.3	18.3	3.9	3.9
		%			16.	25.8	33.6	37.7	30.	30.

* This tadpole was abnormal both in behavior and in form of the regenerated part so is not included in the total.

TABLE 9 EXPERIMENT 5

Carbon dioxid production in Bases - KOH

No.	1	2	3	4	5	6	7
Wt.of 5 tadpoles	4.7	5.1	5.0	5.0	4.9	4.6	4.6
Normal CO_2 Production in Distilled Water							
date							
3/9/18	29.4	34.7	33.9	31.9	34.4	26.2	34.5
3/10	27.1	32.7	30.8	32.6	31.9	23.1	28.3
3/11	26.5	27.2	25.8	30.7	28.5	*14.6	26.3
3/12	24.8	24.1	26.8	27.5	24.5	15.0	24.1
3/13	26.5	30.4	30.7	32.2	30.3	14.3	26.4
3/14	28.1	28.2	27.1	28.0	26.1	16.9	24.8
Total CO_2	162.4	177.3	175.1	182.9	175.7	110.1	164.4
Av.Daily CO_2	27.06	29.65	29.18	30.48	29.28	18.35	27.4
CO_2 per gm.day	5.76	5.81	5.83	6.09	5.97	4.0	5.95
CO_2 Production in KOH							
cc 0.01N KOH in 400 cc H_2O	10	15	20	25	30	35	0
3/15	28.1	26.4	30.4	33.5	38.9	44.0	19.8
3/16	21.8	26.2	24.4	29.2	31.0	33.9	14.7
3/17	26.1	28.9	34.1	36.5	30.8	dead	21.0
3/18	25.2	26.8	31.0	33.5	dead		18.6
3/19	23.0	30.9	30.7	31.4			17.4
Total CO_2	124.2	139.2	150.6	164.1	100.7	77.9	91.5
CO_2 per day	24.84	27.84	30.12	32.82	33.6	38.95	18.3
CO_2 per gm.day	5.28	5.46	6.02	6.56	6.85	8.46	4.0
% of Normal CO_2	91.6	93.9	103.2	107.6	114.7	212.3	66.8

* The $Ba(OH)_2$ used to absorb the CO_2 became mixed with the water When found the tadpoles were almost dead and from that time they were abnormal.

cc.K
in 2
cc.H

10

15

20

25

0

TABLE 10 EXPERIMENT 5

Regeneration in Bases - KOH

cc.KOH in 200 cc.H_2O	Tad. no.	Length in mm.	Length removed in mm.	Length Regenerated										Approximate PH	
	begun 3/19/18			3/23	3/25	3/27	3/30	4/3	4/5	4/9	4/13	4/17	4/21	initial	after 48 hrs.
10	1	48	11.2	1.1	2.2	3.5	5.0	5.0	6.0	7.0	7.2	7.0	7.0	9	6.7
	2	45	9.8	1.0	2.0	2.9	3.6	4.0	4.3	4.7	5.3	5.1	5.2		
	3	44	10.3	1.2	1.8	2.6	3.7	4.5	4.5	5.0	4.9	4.9	4.8		
	4	46	10.0	1.0	2.0	3.0	3.6	4.0	4.2	5.0	5.0	4.8	5.0		
	total		41.3	4.3	8.0	12.0	15.9	17.5	19.0	21.7	22.4	21.8	23.0		
	%			10.4	19.3	29.0	38.	42.	46.	52.5	54.	52.5	55.7		
15	1	51	9.8	1.0	1.4	2.1	2.9	3.3	3.5	5.0	5.0	5.0	5.0	9.6	6.8
	2	48	11.1	0.9	1.6	2.1	3.8	3.7	5.1	3.5	3.5	3.1	3.1		
	3	50	10.1	1.1	2.0	2.9	3.4	4.6	4.5	5.1	5.1	5.0	5.0		
	4	46	11.1	0.8	1.8	2.4	2.9	4.1	4.2	3.5	3.3	3.0	3.2		
	5	44	10.3	1.1	2.0	2.4	3.5	3.0	3.0	4.5	4.7	4.5	4.5		
	total		52.4	4.9	8.8	11.9	16.5	18.7	20.3	21.6	21.6	20.6	20.8		
	%			9.4	16.8	23.	32.	36.	39.	41.	41.	40.	40.		
20	1	48	10.1	0.5	1.6	2.0	3.6	3.6	5.0	5.2	4.9	5.1	5.0	10	6.9
	2	49	11.0	0.7	1.3	2.3	3.5	3.8	4.2	5.0	5.0	3.9	4.0		
	3	48	11.2	1.0	1.8	2.8	3.0	3.2	4.6	4.1	3.9	4.2	4.2		
	4	50	10.5	0.9	1.3	2.2	3.1	3.3	3.6	4.2	4.3	dead			
	5	40	10.5	1.0	1.7	2.5	3.2	3.2	4.3	4.8	4.8	dead			
	total		53.3	4.1	7.7	11.8	16.4	17.1	21.7	23.3	22.9	13.2	13.2		
	%			9.5	14.4	22.1	26.	32.	40.	43.	43.	40.8	40.8		
25	1	46	10.0	0.5	1.7	2.8	3.2	3.3	3.6	3.7	3.7	dead			
	2	48	11.2	1.0	1.5	2.1	2.2	3.3	3.8	3.2	3.3	dead			6.9
	3	46	10.3	1.0	1.3	1.9	2.5	3.2	3.5	4.4	4.5	4.5	dead		
	4	42	10.2	0.6	1.6	2.0	2.5	3.1	3.3	3.3	3.4	3.3	3.3		
	total		41.7	3.1	6.1	8.8	11.0	12.9	14.2	14.6	14.9	7.7	3.3		
	%			7.4	14.3	21.	26.	31.	34.	35.	36.	37.5	32.		
0	1	49	10.3	1.0	1.8	2.7	4.0	4.8	5.4	4.9	5.5	5.5	5.6	7	6.7
	2	47	11.0	1.1	2.0	2.6	3.7	4.4	4.6	5.3	5.3	5.7	5.5		
	3	44	10.1	1.1	1.8	2.7	3.6	4.0	5.0	5.0	5.0	5.0	4.6		
	4	49	10.8	1.0	1.8	2.6	3.9	4.5	4.6	4.4	4.8	4.8	4.1		
	5	47	10.3	1.0	2.0	2.6	3.8	4.2	4.3	5.0	4.2	4.0	5.0		
	total		52.5	5.2	9.4	13.2	19.0	21.9	23.9	24.6	24,8	25.0	24.8		
	%			9.9	18.	25.	36.	41.5	45.	47.	47.	47.	47.		

TABLE 11 EXPERIMENT 6

Carbon dioxid Production in Bases - $Ca(OH)_2$

No.	1	2	3	4	5	6	7
Wt. of Tadpoles	4	4.1	4	4.3	4	3.8	3,7

Normal CO_2 Production in Distilled Water

date	1	2	3	4	5	6	7
3/9/18	29.8	25.7	29.4	*17.3	27.3	28.7	28.9
3/10	26.6	23.0	26.8	19.5	19.7	25.9	25.4
3/11	23.7	22.9	22.7	17.1	20.6	20.4	22.1
3/12	21.8	22.3	21.4	16.1	19.2	19.3	18.4
3/13	29.2	27.2	24.6	21.0	24.6	25.0	17.5
3/14	25.0	21.7	24.7	19.8	20.3	22.5	19.3
Total CO_2	156.1	142.8	148.6	110.8	131.7	141.8	131.6
Av. CO_2 per day	26.01	23.8	24.93	18.47	21.95	23.63	21.93
CO_2 per gm. day	6.5	5.8	6.23	4.3	5.5	6.2	5.95

CO_2 production in $Ca(OH)_2$

$Ca(OH)_2$ in 400 cc. H_2O	10	15	20	25	30	35	0
3/15	25.6	26.1	27.3	30.8	33.2	35.5	19.7
3/16	19.5	20.8	22.4	26.2	19.8	30.0	17.6
3/17	23.5	23.1	28.7	35.2	2 dead	2 dead	14.1
3/18	21.3	22.0	25.9	38.1			13.1
3/19	21.2	23.2	23.1	28.4			10.1
Total CO_2	111.1	115.2	127.4	148.7	53.0	65.5	74.6
Av. CO_2 per day	22.22	23.04	25.5	29.74	26.5	32.75	14.92
CO_2 per gram day	5.55	5.62	6.37	6.91	6.73	7.12	3.24
% of Normal CO_2	85.4	96.8	102.3	161.	120.7	134.	68.

* Water contaminated by $Ba(OH)_2$.

[illegible]

[illegible]

[illegible]

TABLE 12 EXPERIMENT 6

Regeneration in Bases - Ca(OH)$_2$

Ca(OH)$_2$ in 400 cc.H$_2$O	Tad. no.	Length in mm.	Lemgth removed in mm.	Length Regenerated in mm.										Approximate PH	
	begun 3/19/18			3/23	3/25	3/27	3/30	4/3	4/5	4/9	4/13	4/17	4/20	initial	after 48 hrs.
10	1	50	10.5	1.2	1.6	2.2	3.2	3.8	4.1	4.1	4.1	4.1	4.1	9	6.8
	2	45	9.9	0.9	2.0	2.8	4.0	4.5	5.2	4.4	5.5	5.5	5.6		
	3	43	11.0	1.3	2.2	3.0	3.2	4.8	4.5	5.0	5.0	5.0	5.0		
	4	43	10.0	0.8	2.0	2.5	3.7	4.3	4.4	5.3	5.3	5.3	5.0		
	5	41	10.4	1.1	1.8	3.0	3.8	4.4	4.9	5.0	5.0	5.0	5.0		
	total		51.8	5.3	9.6	13.5	17.9	21.8	23.1	23.8	24.9	24.9	24.7		
	%			10.2	18.3	26.	34.5	42.	45.	46.	48.	48.	48.		
15	1	43	10.5	1.1	1.9	2.9	3.6	5.0	4.8	4.9	4.9	4.8	5.0	9.6	7
	2	43	11.0	1.1	2.0	3.0	3.8	4.4	5.0	4.8	5.0	5.0	4.7		
	3	47	11.0	0.8	2.0	3.0	3.8	4.4	4.6	5.5	4.7	4.7	4.5		
	4	46	10.0	1.0	2.1	3.1	3.9	3.9	4.1	5.0	5.3	4.9	4.9		
	5	43	10.1	1.0	1.9	2.8	3.4 V	4.0	4.5	3.8	4.2	4.0	4.0		
	total		52.6	5.0	9.9	14.8	18.5	21.7	23.0	24.0	24.1	23.4	22.9		
	%			9.5	18.9	28.	35.	41.5	44.	45.	46.	44.4	43.5		
20	1	46	10.0	0.7	2.0	2.7	3.3	4.0	4.7	4.0	4.5	dead		10	6.9
	2	42	10.6	1.0	1.7	2.7	3.0	4.0	4.1	5.0	5.1	4.9	5.0		
	3	44	10.0	1.0	1.8	2.7	3.6	4.4	3.8	4.1	4.2	4.3	4.3		
	4	41	10.8	1.0	1.8	2.2	3.1	4.0	4.1	4.1	4.1	4.0	4.0		
	5	41	10.0	1.0	1.8	2.3	3.2	3.7	4.2	3.8	3.9	dead			
	total		51.4	4.7	9.1	12.6	16.2	20.1	20.9	21.0	21.8	13.2	13.0		
	%			9.2	17.7	24.5	31.5	29.	40.5	41.	42.	41.8	41.		
25	1	45	10.4	0.9	1.7	2.5	3.0	4.4	4.5	3.7	3.8	3.8	4.0		7.2
	2	44	10.1	1.0	1.5	2.2	2.2	3.0	4.1	4.1	4.1	dead			
	3	43	10.0	1.0	1.8	2.3	3.1	3.7	4.5	4.5	4.5	4.5	4.5		
	4	45	10.0	0.5	1.7	2.7	3.3	3.8	3.7	4.1	4.5	dead			
	5	46	10.2	0.8	1.5	2.2	3,0	3.5	4.0	4.5	4.7	4.7	4.5		
	total		50.7	4.2	8.2	11.9	14.6	18.4	20.8	20.9	21.6	13.0	13.0		
	%			8.3	14.2	23.5	29.	36.	41.	41.	42.	42.	42.		
30	1	43	10.9	0.8	1.3	2.0	2.0	2.2	2.7	2.8	2.8	dead			
	%			7.3	11.9	18.3	18.3	20.	25.	26.	26.				
0	1	45	10.0	1.0	1.7	2.7	3.8	4.1	4.2	4.4	5.0	5.0	4.9	7	6.7
	2	49	11.1	1.0	2.0	2.7	3.2	4.5	4.5	5.0	5.0	5.0	4.0		
	3	42	10.0	1.4	2.1	2.8	3.3	4.5	4.5	4.0	4.2	4.2	4.7		
	4	42	10.5	1.2	1.9	2.2	3.0	3.2	3.8	4.4	4.4	3.8	4.4		
	total		52.4	5.7	9.5	13.3	16.9	21.0	21.7	5.0	4.0	4.4	3.8		
	%			10.9	18.2	25.4	32.3	40.	41.5	22.8	22.6	22.4	21.8		
										44.5	44.	44.	41.5		

TABLE 13 EXPERIMENT 7

Regeneration in Bases - NaOH

NaOH per liter	Length in mm.	Length removed in mm.	Length in mm. Regenerated						
	begun 4/16/18		4/20	4/22	4/23	4/26	4/28	4/30	5/4
0	37	11.0	1.0	1.6	2.7	3.1	3.5	3.7	4.1
	38	9.9	1.0	2.0	3.0	3.3	3.5	3.9	4.8
	37	10.9	0.8	2.0	3.0	3.7	4.1	4.4	4.9
	37	10.3	1.0	2.0	3.0	3.5	3.8	3.9	4.5
	35	10.3	1.0	1.9	2.8	3.2	4.0	4.1	4.3
	total	52.4	4.8	9.5	14.5	16.8	18.9	20.0	22.6
	%		9.2	18.2	27.7	32.	36.	38.	43.
25	37	10.1	0.6	1.6	2.2	2.2	3.0	3.0	3.0
	38	10.4	0.8	1.5	2.0	2.5	2.6	3.7	3.4
	38	10.4	0.8	1.2	2.1	2.7	3.1	3.1	4.1
	36	11.0	0.7	1.6	2.1	2.8	3.5	3.0	3.1
	39	11.0	0.7	1.6	1.9	2.5	3.0	3.2	3.6
	total	52.9	3.6	7.5	10.3	12.7	15.2	16.0	17.2
	%		6.8	14.2	19.5	24.	29.	30.	32.6
50	39	11.0	dead 4/19						
	40	10.0	dead 4/20						
	35	10.0	" "		no regeneration				
	41	10.2	" "						
	35	9.3	dead 4/19						
0	39	11.0	1.0	2.1	3.0	3.2	3.9	3.9	4.2
	40	10.2	0.8	2.0	3.0	3.8	3.5	4.0	4.5
	37	10.3	1.0	2.2	2.9	3.4	4.0	4.0	4.2
	35	10.6	1.0	1.8	3.0	3.3	3.9	3.9	4.4
	36	9.7	1.1	1.9	2.6	3.0	3.6	4.2	4.2
	total	51.8	4.9	10.0	14.5	16.7	18.9	20.0	21.5
	%		9.5	19.3	28.	32.	36.5	38.6	41.5
25	40	11.2	1.0	1.6	2.0	2.3	2.8	2.6	3.0
	38	10.2	0.8	1.1	1.2	1.5	dead		
	39	10.8	0.7	1.0	1.8	2.0	2.9	3.0	3.0
	35	9.4	0.7	1.1	1.9	2.0	2.6	2.6	3.0
	35	9.9	0.6	1.3	2.0	2.3	2.5	2.9	3.1
	tOtal	51.5	2.9	6.1	8.9	10.1	10.8	11.1	12.1
	%		5.64	11.9	17.3	19.6	26.	27.	29.3
50	38	9.8	dead 4/19						
	39	10.2	dead 4/20						
	38	10.2	" "		no regeneration				
	35	11.0	dead 4/19						
	37	10.1	" "						

Approximate PH of Solutions

	initial	after 24 hours
0	7	6.5 to 6.6
25	8.9 to 9	8 to 8.2
50	10 or more	9 to 9.6(4/20)

TABLE 14 EXPERIMENT 8

Regeneration in Acid - H_3PO_4

H_3PO_4 in 200 cc.H_2O	Length in mm.	Length removed in mm.	Length Regenerated in mm. Begun 4/4/18 4/10	4/14	4/16	4/18	4/21	4/24	4/26	4/30	5/4	Approximate PH of Solutions initial	after 24 hours.
25	50	13.1	1.4	2.2	4.0	4.5	dead					3.4	6.7
	53	13.2	1.0	2.8	4.1	3.9	"						
	46	13.5	1.6	3.5	3.9	4.3	"						
	44	12.0	1.0	2.0	3.8	4.2	"						
	total	51.8	5.0	10.5	15.8	16.9							
	%		9.6	20.1	30.3	32.6							
20	48	13	1.2	3.1	3.6	4.1	dead					4.0	5.3
	50	12.9	1.8	3.1	3.6	4.0	"						
	39	12	1.7	3.2	3.4	4.0	"						
	47	13.1	1.8	3.7	4.2	4.8	"						
	total	51.0	6.5	12.7	13.1	14.8							
	%		12.7	26.	29.	33.							
15	50	13	1.5	3.2	4.1	4.5	4.1	4.1	4.0	4.0	4.0	4.8	6
	45	12.2	1.8	3.6	4.0	4.0	4.8	dead					
	39	12.2	1.9	3.5	3.9	4.8	5.1	"					
	43	13 B	1.6	3.2	4.5	4.7	5.1	"					
	TOTAL	50.4	6.8	13.5	16.5	18.0	19.0	4.1	4.0	4.0	4.0		
	%		13.5	27.	33.	36.	38.	32.	32.	636	32.		
10	51	13	1.5	3.0	3.6	4.7	5.0	5.0	5.0	5.7	5.7	5.7	6.3
	43	12.2	2.0	3.2	4.5	4.3	4.9	4.9	5.0	5.5	5.3		
	43	12.8	2.0	3.6	4.1	4.5	4.7	5.0	5.0	5.0	5.0		
	42	13.7	1.5	3.1	4.1	4.8	5.2	5.2	5.3	5.7	5.7		
	total	51.7	7.0	12.9	16.3	18.3	19.8	20.1	20.8	21.9	21.7		
	%		13.5	25.	31.6	35.4	38.	39.	41.	43.	42.		
5	51	13.1	1.1	3.0	3.8	4.1	4.7	4.7	5.0	5.1	5.1	6.3	6.4
	50	12.9	1.9	3.2	4.1	4.3	4.8	4.9	5.2	5.2	5.0		
	44	14	2.0	3.4	4.5	4.9	5.0	5.5	5.8	6.0	6.0		
	38	11	1.5	2.5	3.0	3.8	3.8	3.8	4.0	4.1	4.1		
	total	51.0	6.5	12.1	15.4	17.1	18.3	18.9	20.0	20.5	20.1		
	%		12.7	24.	28.4	34.	36.	37.	39.	40.	39.		
0	53	13	1.5	3.2	3.9	4.4	5.0	5.0	5.5	5.5	5.5	7.0	6.7
	51	12.7	1.9	3.1	3.5	4.0	4.6	4.7	4.9	5.0	5.0		
	45	12.1	1.9	3.0	3.8	4.2	4.9	4.9	5.0	5.0	5.0		
	39	12.3	1.9	4.0	4.8	5.2	5.5	5.5	6.1	6.1	6.0		
	total	50.1	7.2	13.3	16.0	17.8	20.0	20.1	21.5	21.6	21.5		
	%		14.3	26.5	32.	35.5	40.	40.4	43.	43.	43.		
KH_2PO_4										6.5	6.5	7.0	6.7
+	50	13.8	1.4	3.7	4.7	4.5	5.2	5.8	6.1	5.2	5.2		
Na_2HPO_4	51	12.3	1.9	3.1	4.0	4.3	4.9	5.0	5.0	5.1	5.1		
	43	12.4	1.8	3.1	3.9	4.4	4.7	4.7	4.9	6.2	6.3		
	39	13	2.0 m	3.5	4.1	4.9	5.3	5.7	6.0	23.0	23.0		
	total	51.5	7.1	13.4	16.7	18.1	20.1	21.2	22.0	45.	45.		
			13.8	26.	30.5	35.	39.3	41.5	43.				

TABLE 14a EXPERIMENT 8

Regeneration in Bases - NaOH

NaOH in 200 cc.H20	Length in mm.	Length removed in mm.	Length Regenerated in mm. Begun 4/4/18 4/10	4/14	4/16	4/18	4/21	4/24	4/26	4/30	5/4	Approximate PH of Solution initial	after 24 hours.
0	51	13.1	1.3	3.1	3.9	4.9	5.2	5.8	6.2	6.5	6.5	7	6.7
	44	13	1.8	3.1	4.3	4.9	5.2	5.6	6.0	6.1	6.4		
	49	13	1.9	3.2	4.2	5.0	5.0	5.5	5.8	5.8	5.8		
	39	11.3	2.0	3.4	4.0	4.5	4.9	4.9	5.2	5.2	5.2		
	total	50.4	7.0	12,8	16.4	19.3	20.3	21.8	23.3	23.6	23.9		
	%		13.9	25.4	32.6	38.	40.	43.	47.	47.	47.		
5	50	12.9	2.0	3.3	4.7	5.2	5.3	6.6	6.0	6.4	6.4	9.2	6.9
	48	13.4	1.8	3.1	4.2	5.0	5.2	5.8	5.9	5.9	5.9		
	42	12	1.9	3.5	4.1	4.8	5.0	5.6	5.6	5.6	5.6		
	44	12.5	1.8	3.3	4.0	4.6	5.0	5.0	5.3	5.3	5.3		
	total	50.8	7.5	13.2	17.0	19.6	20.5	22.4	22.8	23.2	23.2		
	%		14.7	26.	33.5	38.6	39.6	44.	45.	46.	46.		
10	50	13.1	1.1	3.6	3.8	4.5	4.9	5.1	5.8	5.8	5.8		7.1
	45	12.9	1.3	3.4	3.6	4.0	4.0	4.5	5.0	5.0	5.0		
	45	12.	1.8	2.8	4.0	3.9	4.1	4.5	4.5	4.9	4.9		
	38	11	1.8	3.0	3.3	4.2	4.6	5.0	5.0	5.2	5.2		
	total	49.	6.0	12.8	14.6	16.6	17.6	19.1	20.3	20.9	20.9		
	%		12.4	26.	30.	34.	36.	39.	41.5	42.5	42.5		
12.5	54	13	1.2	2.7	3.2	3.1	disappeared						
	47	13.4	1.8	2.9	3.6	4.0	4.3	4.2	4.8	5.6	5.0		7.2
	42	12.5	1.1	3.4	4.0	4.0	4.5	4.9	5.0	5.3	5.3		
	38	12	1.1	3.0	3.1	3.5	3.7	3.8	4.1	4.2	4.2		
	tptal	50.9	5.2	12.0	13.9	14.6	12.4	12.7	14.9	14.5	14.5		
	%		10.3	23.6	27.4	29.	33.	36.	39.	38.	38.		
15	52	13.6	1.6	3.0	3.7	3.8	3.3	3.7	4.0	4.9	4.8		7.4
	49	12.2	1.4	2.7	3.1	3.2	3.9	4.3	4.5	4.0	4.0		
	44	13	1.1	2.8	3.1	3.5	3.5	3.5	3.7	4.1	4.1		
	42	12	1.7	2.8	3.5	3.9	4.2	4.3	4.6	4.8	4.8		
	TOTAL	50.8	5.8	11.3	13.4	14.4	14.9	15.6	16.8	17.8	17.7		
	%		11.4	22.2	26.4	28.4	29.4	30.7	33.	35.	35.		
17.5	51	13.7	1.6	2.9	3.4	3.9	4.0	4.2	4.7	4.8	4.5		5.5
	45	12.5	1.6	3.1	3.4	3.3	3.4	3.8	4.3	4.5	4.5		
	40	12	1.5	2.9	3.1	3.6	4.1	4.7	4.7	4.9	5.0		
	40	12.8	1.6	2.5	3.1	3.7	4.0	dead					
	total	51	6.3	11.3	13.0	14.5	15.5	12.7	13.7	14.2	14.0		
	%		12.3	22.3	25.5	28.4	30.4	33.	36.	37.	37.		
20	50	13.5	2.0	2.8	3.3	3.4	3.6	3.7	3.6				
	47	14	1.1	3.0	3.0	3.1	3.3	4.0	4.0	4.1	4.1		7.5
	40	12.5	1.3	2.7	3.1	3.5	3.5	3.6	3.7	3.7	3.7		
	41	12	1.2	2.9	3.2	3.7	4.2	4.3	4.3	4.5	4.0		
	total	52.0	5.6	11.4	12.6	13.7	14.6	15.6	15.6	12.3	11.8		
	%		10.8	22.	24.2	26.4	28.	30.	30.	32.	31.		

TABLE 15 EXPERIMENT 9

Regeneration in Acid - HNO_3

cc, HNO_3 in 200 cc. H_2O	Tad. no.	Length in mm.	Length removed in mm.	Length Regenerated in mm.										Approximate PH of Solution		
Date - Begun 3/ 9/18 - - -				3/13	3/17	3/20	3/23	3/25	3/27	3/29	3/31	4/2	4/4	initial	after 24 hrs.	after 28 hrs,
	1	43	10.4	1.0	2.7	3.6	4.4	4.5	4.7	5.0	5.2	5.1	5.1	7	6.8	6.8
0	2	40	11.0	1.0	3.3	3.6	4.3	4.6	4.5	5.0	5.0	5.0	5.0			
	3	39	10.7	1.1	2.9	killed by accident.										
	4	44	10.9	1.0	3.1	4.1	4.4	4.6	4.4	4.8	4.8	5.0	5.0			
Total			43.0	4.1	12.0	15.3	13.1	13.7	13.6	14.8	15.0	15.1	15.1			
%				9.5	28.0	35.0	40.	42.5	42.5	46	46.5	46.5	46.5	5.4	6.4	6.4
	1	44	10.9	1.0	3.4	4.1	4.5	4.5	4.5	4.5	4.5	4.5	4.5			
5	2	43	10.5	1.1	2.9	3.8	4.3	4.5	4.8	5.0	5.0	5.0	5.0			
	3	39	10.7	1.0	2.5	3.5	4.3	5.0	4.5	4.8	4.8	5.3	5.2			
	4	40	11.0	0.8	3.0	3.5	4.1	4.3	4.3	4.6	4.7	4.8	4.8			
Total			43.1	3.9	11.8	14.9	17.2	18.3	18.1	18.9	19.0	19.6	19.5			
%				9.1	27.5	34.8	40.0	42.3	42.0	44.	44.	45.	45.			
7.5	1	38	10.6	1.1	3.0	3.5	3.9	4.3	4.1	4.1	4.1	4.1	4.1	4.5	6	6.2
	2	36	10.0	1.0	3.2	2.6	4.3	4.1	4.1	4.3	4.4	4.4	4.2			
	3	42	10.2	0.7	2.9	3.2	4.0	4.3	4.5	4.7	4.5	4.7	4.3			
	4	34	11.0	1.0	2.1	3.2	4.2	4.1	4.1	4.5	4.7	4.5	4.7			
Total			41.8	3.8	11.2	13.5	16.4	16.8	16.8	17.6	17.7	17.7	17.5			
%				9.1	26.8	32.0	39.0	40.0	40.0	42.	42.	42.	42.			
10	1	39	10.4	1.0	3.0	3.6	4.1	4.5	4.4	4.4	4.6	4.7	4.6	3.8	5.4	6.1
	2	41	10.8	0.9	2.6	3.2	3.8	3.8	4.0	4.3	4.3	4.1	4.1			
	3	45	11.0	0.8	2.7	3.6	4.1	4.1	3.9	4.5	4.5	4.5	4.5			
	4	40	10.5	1.1	2.4	3.0	3.8	4.0	3.8	4.0	4.0	4.0	4.0			
Total			42.7	3.8	10.5	13.4	15.8	16.4	16.1	17.2	17.4	17.3	17.2			
%				8.9	24.6	31.4	37.0	38.4	38.4	40.	40.	40.	40.			
	1	43	10.0	1.1	2.8	3.5	3.4	3.5	4.0	4.0	3.9	3.6	3.6	3.4	4.9	5.5
12.5	2	39	10.7	1.0	3.1	3.6	3.7	3.8	3.3	3.5	3.6	3.6	dead			
	3	36	10.0	0.7	2.4	3.0	4.1	4.6	5.0	5.0	5.0	5.0	dead			
	4	38	11.2	0.8	2.4	2.9	3.4	3.8	3.5	3.7	3.7	3.6	3.6			
Total			41.0	3.6	10.7	13.0	14.6	15.7	15.8	16.2	16.2	15.8	7.2			
%				8.6	25.5	31.	35.	37.	38.	38.5	38.5	38.0	34.			
	1	47	10.0	0.9	2.3	2.3	2.2	2.3	dead							
15	2	40	10.0	0.5	1.4	2.1	2.1	dead						3.2	4.4	5.0
	3	38	10.2	0.6	1.9	dead										
	4	37	10.8	0.7	2.1	2.3(T)	2.3	2.5	2.8)							
Total			41.0	2.7	7.7	6.7	2.3	2.3		dead						
%				6.6	18.8	22.0	23.0	23.0								
	1	41	9.0	0.7	dead									3.1	4.1	5.0
17.5	2	40	10.2	0.7	1.9	[illegible]	dead									
	3	40	10.0	0.5	1.2	dead										
	4	41	12.0	0.4	1.0(T)	1.0	dead									
Total			41.2	2.3	4.1											
%				5.1	12.7	(other three didd								3.0	3.4	5.0
20	1	41	10.8		1.1	dead	without regeneration									

TABLE 16 EXPERIMENT 10

Regeneration in Acid - HBr.

cc.HBr in 200 cc.H_2O	Tad. no.	Length in mm.	Length removed in mm.	Length Regenerated in mm.								Approximate PH of Solution
	Begun	3/12/18	- - - -	-3/16	3/19	3/23	3/27	3/29	3/31	4/2	4/6	
0	1	50	12.0	1.1	3.2	4.4	4.8	5.2	5.4	5.5	5.5	
	2	47	12.5	1.1	2.7	3.8	4.5	4.9	5.0	5.0	4.8	7
	3	44	10.0	1.0	2.3	3.3	3.5	4.0	4.0	4.0	4.1	
Total			34.5	3.2	8.2	11.5	12.8	14.1	14.4	14.5	14.4	
%				9.3	24.	33.	37.	41.	42.	42.	42.	
5	1	60	14.0	1.5	1.8	3.9	4.5	5.0	5.3	5.3	5.3	
	2	45	10.5	1.1	2.6	3.8	4.1	4.2	4.2	4.2	4.2	5.5
	3	45.	10#3	1.1	2.5	3.6	4.2	4.2	4.3	4.3	4.2	
Total			34.8	3.9	7.9	11.3	13.0	13.4	13.8	13.8	13.7	
%				10.6	22.7	32.4	37.	38.5	39.3	39.3	39.	
7.5	1	51	12.0	1.1	3.0	4.1	4.9	5.1	5.1	5.1	5.1	
	2	45	11.1	1.0	2.2	3.5	4.0	4.1	4.2	4.2	4.2	4.6
	3	45	11.3	1.1	2.7	3.6	4.4	4.6	4.7	4.8	4.8	
Total			34.4	3.2	7.9	11.2	13.3	13.8	14.0	14.1	14.1	
%				9.3	23.	32.5	39.	40.	41.	41.	41.	
10	1	54	13.0	---	2.0	3.4	3.8	4.0	4.0	4.0	4.2	
	2	46	11.6	1.0	2.1	3.5	4.4	4.6	4.9	4.8	4.8	3.9
	3	42	9.0	0.8	2.2	3.2	3.7	3.7	4.0	4.0	3.7	
Total			33.6	1.8	6.3	10.1	11.9	12.3	12.9	12.8	12.7	
%				8.8	18.7	30.	35.4	36.6	38.	38.	38.	
12.5	1	53	12.8	0.8	2.3	2.9	3.5	3.5	3.5	3.6	3.6	
	2	46	11.3	0.9	2.2	3.2	4.0	4.2	4.2	4.2	4.2	3.4
	3	44	11.0	0.8	1.9	2.8	3.5	3.7	3.8	4.0	3.8	
Total			35.1	2.5	6.4	8.9	11.0	11.4	11.5	11.8	11.6	
%				7.1	18.2	25.	31.4	32.5	33.	33.	33.	
15	1	50	11.9	0.9	2.0	2.7	3.0	dead				
	2	48	12.3	0.7	2.0	2.7	3.0	3.0	3.0	3.0	dead	3.3
	3	44	10.9	0.9	1.8	2.4	3.1	3.1	dead			
Total			35.2	2.5	5.8	7.8	9.1	6.1	3.0	3.0		
%				7.1	16.5	22.0	26.	?26.	24.4	24.4		
17.5	1	47	12.0		1.0(T)	1.8	dead					
	2	46	11.6		dead							3.2
	3	45	10.1		〃							
Total					1.0							
%					8.3							
20	All died without regeneration.											3.1

Regenera

cc.H_2SO_4
in 200
cc.H_2O
Date - I
0

Total
%
5

Total
%
7.5

Total
%
10

Total

12.5

Total
%
15

Total
%
17.5

Total
%
20

Total
%
(T) in

TABLE 17 EXPERIMENT 11

Regeneration in Acids - H_2SO_4

cc.H_2SO_4 in 200 cc.H_2O	Tad. no.	Length in mm.	Length removed in mm.	Length Regenerated in mm.										Approximate PH of solution.		
Date - Begun		3/9/18-- - - -		3/13	3/17	3/20	3/23	3/25	3/27	3/29	3/31	4/2	4/6	initial	after 24 hrs.	after 48 hrs.
0	1	41	11.0	1.0	3.0	4.0	5.0	5.0	4,8	4.8	4.8	5.0	4.0			
	2	43	10.7	1.0	3.5	4.3	4.7	4.9	5.1	5.3	5.0	5.0	5.0	7	6.8	6.8
	3	40	10.0	1.0	3.4	4.0	4,7	4.9	5.0	5.3	5.5	5.2	5.5			
	4	40	10.9	1.0	3.3	3.6	4.5	4.6	4.6	4.8	4.8	5.0	4.0			
Total			42.6	4.0	13.2	15.9	19.2	19.3	19.5	20.2	19.9	20.2	20.4			
%				9.4	31.0	37.0	45.0	45.0	46.	48.	47.	48.	49.			
5	1	46	10.6	1.2	3.6	4.0	4.5	4.5	4.5	4.8	4.8	4.7	5.0			
	2	43	10.2	0.8	3.0	3.8	4.4	4.3	4.4	4.6	5.0	5.0	5.1			
	3	40	11.0	1.0	2.9	3.5	4.5	5.0	4.8	5.0	5.2	5.2	4.7	5.5	6.4	6.5
	4	38	11.0	0.9	3.0	3.9	5.1	5.1	5.1	5.3	5.3	5.3	5.3			
Total			42.8	3.9	12.5	15.2	18.5	18.9	18.8	19.7	20.2	20.2	20.1			
%				9.1	28.6	35.5	43.	44.	44.	46.	48.	48.	48.			
7.5	1	36	10.0	1.1	3.0	3.8	4.6	5.0	5.0	5.1	5.0	5.0	5.0	4.5	6.0	6.3
	2	29	11.0	1.1	2.9	3.8	4.1	4.7	4.3	4.9	4.9	4.9	5.0			
	3	36	9.4	1.0	2.1	3.0	3.5	3.5	3.7	4.0	4.0	4.0	4.0			
	4	388	10.1	0.9	2.2	3.2	3.0	3.0	3.4	3.6	3.6	3.6	3.5			
Total			40.5	4.1	10.2	13.8	15.2	16.4	16.4	17.6	17.5	17.5	17.5			
%				9.9	25.2	34.0	37.	40.	40.5	43.5	43.0	43.0	43.0			
10	1	43	10.1	0.8	3.0	3.4	4.0	4.1	4.3	4.5	4.5	4.5	4.5	3.9	5.5	6.1
	2	39	10.0	0.8	2.5	3.8	4.1	4.5	4.1	4.3	4.4	4.4	4.4			
	3	37	10.2	0.9	2.3	3.2	3.3	3.3	3.8	4.1	4.1	4.1	4.1			
	4	38	11.0	0.9	2.2	3.2	4.2	4.3	4.0	4.5	4.4	4.4	4.4			
Total			41.3	3.4	10.0	13.6	15.6	16.2	16.2	17.4	17.4	17.4	17.4			
				8.2	24.2	33.0	37.8	39.0	39.	42.	42.	42.	42.			
12.5	1	42	10.0	0.6	2.2	3.0	4.0	4.1	4.0	4.5	4.5	4.5	4.0	3.4	4.9	5.5
	2	43	10.0	1.2	2.6	3.0	3.8	4.0	4.0	4.1	4.2	4.0	4.5			
	3	37	10.5	1.0	2.8	3.4	3.5	3.5	3.5	3.7	4.0	4.0	4.0			
	4	36	10.3	0.8	2.0	2.4	4.0	4.0	4.0	4.1	4.5	4.5	4.5			
Total			40.8	3.6	9.6	11.8	15.3	15.6	15.5	16.4	17.2	17.0	17.0			
%				8.8	23.5	29.0	37.7	28.0	38.	40.	40.5	40.5	40.5			
15	1	40	10.0	1.0	2.8	3.1	4.0	4.1	3.7	3.7	3.7	3.7	3.7(T)	no further growth		
	2	41	10.9	0.8	1.7	3.0	3.7	3.8	3.5	3.6	3.4	dead		3.3	4.4	5.0
	3	41	11.0	0.8	2.3	3.0	3.4	3.6	3.6	3.6	3.6	3.6	dead			
	4	35	10.2	0.9	2.0	2.2	2.5	2.6	3.0	3.0	3.0	3.0	dead			
Total			42.1	3.5	8.8	11.3	13.6	14.1	13.8	13.9	13.7	10.3	3.7			
%				8.3	21.0	27.0	32.3	33.0	33.	33.	33.	33.				
17.5	1	39	11.0	1.0	1.6	dead										
	2	43	10.4	0.5	1.2	1.3	1.3	1.3	dead					3.2	not determined	
	3	36	10.6	1.0	1.7	1.7(T)	2.5	3.0	3.0	3.1	dead					
	4	36	10.1	0.7	1.1	1.1	dead									
Total			42.1	3.2	5.6	4.1	1.3	1.3								
%				7.6	13.3	13.2	12.5	12.5								
20	1	40	10.4	0.7	dead											
	2	38	11.0	0.3	1.3(T)	1.3	1.7	2.0								
	3	36	10.0	0.5	1.5	dead			1.8	2.1	2.1	2.3	2.3	3.0		
	4	35	10.1	1.0	dead											
Total			41.5	2.5	2.8											
%				6.0	13.0											

(T) indicates tadpole was transferred to neutral water

Reger

cc.H
in 2
cc.H

Date

0

Total
%

5

Total
%

7.5

Total
%

10

Total
%

12.5

Total
%

15

Total
%

TABLE 18 EXPERIMENT 12

Regeneration in Acids - H_3PO_4

cc.H_3PO_4 in 200 cc.H_2O	Tad. no.	Length in mm.	Length removed in mm.	Length Regenerated in mm.										Approximate PH of Solution		
Date - Begun		3/9/18	- - - -	3/13	3/17	3/20	3/23	3/25	3/27	3/29	3/31	4/2	4/6	initial	after 24 hrs.	after 48 hrs.
0	1	40	10.9	1.0	3.3	3.6	4.5	4.6	4.6	4.8	4.8	5.0	4.9	7	6.8	6.8
	2	40	10.0	1.0	3.4	4.0	5.4	4.8	5.0	5.3	5.5	5.2	5.5			
	3	43	10.7	1.0	3.5	4.3	4.7	4.9	5.4	5.3	5.0	5.0	5.0			
	4	41	11.0	1.0	3.0	4.0	5.0	5.0	4.8	4.8	4.8	5.0	5.0			
Total			42.6	4.0	13.2	15.9	19.2	19.3	19.5	20.2	19.9	20.2	20.4			
%				9.4	31.	37.	45.	45.	46.	48.	47.	48.	49.			
5	1	44	11.0	1.0	3.0	3.6	3.8	4.0	4.7	4.5	4.5	4.6	4.6	6.3	6.6	6.8
	2	32	10.0	1.0	2.8	3.5	4.4	4.3	4.7	5.0	5.0	5.0	5.0			
	3	39	10.6	1.1	3.0	4.0	4.4	4.7	4.8	4.9	4.9	4.8	4.9			
	4	40'	10.9	1.1	3.2	4.1	5.2	5.3	5.7	5.8	5.8	6.0	6.0			
Total			42.5	4.2	12.0	15.3	17.8	18.3	19.9	20.2	20.2	20.4	20.4			
%				9.9	28.2	36.	42.	43.	47.	47.	47.	48.	48.			
7.5	1	42	10.2	0.9	2.5	3.9	4.0	4.1	4.2	4.3	4.2	4.2	4.2	6.	6.4	6.5
	2	44	10.6	1.0	2.8	3.3	4.0	4.2	4.5	5.0	5.0	5.0	5.0			
	3	32	9.0	1.2	2.6	3.5	4.0	4.2	5.0	5.2	5.2	5.2	5.2			
	4	39	10.7	1.1	3.0	3.6	4.3	4.8	4.3	4.7	4.8	4.8	4.7			
Total			40.5	4.2	10.9	14.3	16.3	17.3	18.0	19.2	19.2	19.2	19.1			
%				10.3	27.0	35.3	40.0	43.0	44.5	47.	47.	47.	47.			
10	1	45	12.0	0.8	2.8	3.5	4.2	4.7	5.0	5.5	5.5	5.6	5.5	5.7	6.3	6.4
	2	38	10.0	1.1	3.0	3.1	3.7	3.5	3.5	3.7	4.0	4.0	4.0			
	3	39	11.2	1.0	3.0	4.0	4.2	4.5	4.8	4.8	4.8	5.0	5.0			
	4	37	10.6	1.0	2.7	4.0	3.9	4.0	4.2	4.2	4.3	4.8	4.8			
Total			43.8	3.9	11.5	14.6	16.0	16.7	17.5	18.2	17.6	19.4	19.3			
%				8.9	26.	33.3	36.5	38.	40.	41.5	41.5	44.	44.			
12.5	1	45	10.5	1.1	3.2	3.8	4.5	4.3	4.6	5.1	5.1	5.1	5.1	5.1	6.1	6.3
	2	45	10.9	1.0	3.0	3.7	3.9	4.6	4.6	4.8	4.8	4.8	4.8			
	3	38	10.4	0.9	2.3	3.6	4.2	4.6	4.4	4.4	4.4	4.5	4.4			
	4	40	11.0	1.0	2.4	3.5	4.3	4.1	4.1	3.4	3.4	3.5	3.5			
Total			42.8	4.0	10.9	14.6	16.9	17.6	17.7	17.7	17.7	17.9	17.8			
%				9.3	24.	34.	39.5	41.5	41.5	41.5	41.5	41.5	41.5			
15	1	41	10.0	0.9	2.5	3.6	4.0	4.1	5.0	4.3	4.6	4.7	4.6	4.7	5.9	6.2
	2	36	11.0	1.0	2.7	3.5	4.0	4.5	4.4	5.5	5.5	5.4	5.4			
	3	42	10.3	1.1	3.1	4.1	5.0	5.0	4.6	5.0	4.9	4.9	5.0			
	4	43	11.0	1.0	3.0	4.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0			
Total			42.3	4.0	11.3	15.2	18.0	18.6	19.0	19.8	20.0	20.0	20.1			
%				9.5	27.	36.	42.6	43.7	44.9	47.	47.	47.	47.			

TABLE 18 EXPERIMENT 12 Continued.

Regeneration in Acids - H_3PO_4

cc. H_3PO_4 in 200 cc. water	Tad. no.	Length in mm.	Length removed in mm.	Length Regenerated in mm.										Approximate PH of solution		
D ate -	Begun	3/9/18	- - - -	3/13	3/17	3/20	3/23	3/25	3/27	3/29	3/31	4/2	4/6	initial	after 24 hrs.	after 48 hrs.
17.5	1	40	10.0	1.0	2.6	3.5	4.0	3.9	3.7	4.0	4.0	4.0	4.0	4.2	5.4	5.5
	2	39	10.2	0.5	2.8	3.3	4.0	3.7	4.4	4.4	4.1	4.1	4.1			
	3	35	10.0	1.0	2.9	3.4	3.3	4.1	4.5	4.5	4.2	4.2	4.2			
	4	42	10.2	1.0	2.9	4.0	5.0	5.0	4.5	4.4	4.4	4.5	4.5			
Total			40.4	3.5	11.2	14.2	16.3	16.7	17.1	17.3	16.7	17.3	16.8			
%				8.6	27.8	35.	40.5	41.5	42.	43.	41.	43.	41.			
20	1	43	10.5	0.7	2.2	3.1	3.5	3.9	3.5	3.8	3.8	3.8	3.8	3.9	4.8	5.4
	2	42	10.0	0.9	2.8	3.3	4.0	4.0	3.2	3.8	4.0	4.0	4.0			
	3	37	10.0	0.9	2.8	2.7	3.5	3.8	4.1	4.2	4.2	4.2	4.2			
	4	39	10.9	1.0	3.0	3.9	4.0	4.1	4.0	4.0	4.0	4.0	4.0			
Total			41.4	3.5	10#7	13.8	15.3	16.0	14.8	15.8	16.0	16.0	16.0			
%				8.5	26.	33.4	37.	38.7	36.	38.	38.5	38.5	38.5			
22.5	1	47	10.5	1.0	2.9	4.0	dead		[illegible]	[illegible]						
	2	41	10.4	0.9	2.8	3.6	3.7	4.0	4.0	4.1	4.0	4.0	4.0	3.6	4.7	5
	3	40	11.2	0.9	2.5	3.0	3.2	3.3	3.2	3.2	3.3	3.3	3.3			
	4	43	10.0	1.0	2.4	3.5	dead									
Total			42.1	3.8	10.6	14.1	6.9	7.3	7.2	7.3	7.3	7.3	7.3			
%				9.	25.	33.	32.	34.	33.	34.	34.	34.	34.			
25	1	46	11.0	0.6	2.3	3.2	3.2	3.4	3.2	3.2	3.2	3.2	3.2	3.4	4.4	4.6
	2	43	10.0	1.0	2.6	3.2	dead									
	3	41	10.0	0.9	2.4	2.8	dead									
	4	38	10.0	1.0	3.0	3.8	3.8	3.9	3.5	3.5	3.0	3.0	3.0			
Total			41.0	3.5	10.3	13.0	6.7	6.7	6.7	6.7	6.2	6.2	6.0			
%				8.5	25.	32.	32.	32.	32.	32.	30.	30.	28.5			

TABLE 19 EXPERIMENT 13

A. Normal CO_2 production

No.	gms. Wt. of Tads.	CO_2 produced			Total CO_2	CO_2 per day	CO_2 per gm. per day
date - -		3/29	3/30	3/31			
1	2.9	13.9	11.4	13.8	39.1	13.33	4.6
2	3.0	14.9	17.0	15.5	47.4	15.8	5.26
3	2.9	16.6	15.8	15.5	47.9	15.8	5.45
4	2.9	15.7	14.0	16.1	45.8	15.26	5.26
5	2.6	13.7	11.9	14.8	40.4	13.5	5.2
6	2.7	15.2	13.5	13.3	42.0	14.0	5.18
7	3.0	14.8	11.8	13.5	40.1	13.4	4.5
8	3.0	13.8	13.5	18.2	45.7	15.23	5.07
9	2.6	12.5	13.6	13.3	39.4	13.13	5.05
10	2.5	13.1	10.9	13.3	37.3	12.43	5.0

B. CO_2 production of the same tadpoles in Acids - HCl add HBr

No.	cc.HCl in 400 cc.H_2O	CO_2 produced		Total CO_2	CO_2 per day	CO_2 per gm. day	% of normal CO_2
	date	4/1	4/2				
1	0	12.2	8.7	20.9	10.45	3.6	78.2
2	10	9.3	7.7	17.0	8.5	2.83	53.8
3	20	9.1	7.2	16.3	8.15	2.8	51.5
4	25	8.5	6.1	14.6	7.3	2.5	47.8-2dead
5	30	9.0	5.3	14.3	7.15	2.75	53.5
	cc.HBr in 400 cc.H_2O						
6	10	8.2	5.4	13.6	6.8	2.5	48.5
7	20	7.4	3.6	11.0	5.5	1.83	41.0
8	25	7.1	3.1	10.2	5.1	1.7	33.4
9	30	6.1	2.7	8.8	4.4	1.68	33.4
10	0	11.3	9.4	20.7	10.35	4.14	83.2

CO_2 given as 0.01 N H_2CO_3

TABLE 30 EXPERIMENT 13

Regeneration in Acids - HCl and HBr.

No.	cc.HCl in 400 cc.H$_2$0 date	Length in mm. - Begun	Length removed in Mm. 3/31/18--	Length regenerated in mm. 4/5	4/7	4/9	4/13	4/17	4/21	4/25
1.	0	48	12.4	1.5	2.2	3.0	4.0	4.7	5.0	5.0
		50	11.0	1.0	2.0	2.8	3.4	4.0	4.0	4.0
		42	11.6	1.3	2.6	3.6	4.0	4.2	4.2	4.1
		40	12.0	1.5	2.5	3.1	3.9	4.2	4.3	4.4
	Total		47.0	5.3	9.3	12.5	15.3	17.1	17.5	17.5
	%			11.3	19.8	26.8	32.6	36.0	37.	37.
2	10	53	12.1	0.8	2.0	3.1	disappeared			
		44	12.0	0.9	1.8	3.1	4.0	4.2	4.5	4.5
		44	12.0	1.1	2.0	3.0	3.7	4.2	4.0	4.0
		41	11.0	1.0	2.1	2.8	3.3	3.9	4.2	4.3
	Total		47.1	3.8	7.9	12.0	11.0	12.3	12.7	12.8
	%			8.1	16.0	25.5	31.0	35.	26.	36.
3	20	49	11.0	1.0	2.1	3.0	4.0	4.9	5.0	5.0
		46	12.1	0.9	1.4	2.1	3.2	3.1	3.0	3.0
		45	10.9	0.5	1.5	2.9	2.6	3.8	3.9	3.9
		43	11.7	0.9	1.7	2.8	3.3	3.6	3.6	3.6
	Total		46.6	3.3	6.7	10.7	13.1	15.4	15.5	15.5
	%			7.1	14.7	23.	28.	33.	33.2	33.2
4	25	died without regeneration								
5	30	" " "								
	HBr.									
6	10	50	12.5	1.0	1.8	2.5	3.1	3.5	3.8	4.0
		47	22.3	1.4	2.0	3.0	3.8	4.0	4.0	4.1
		45	11.4	1.4	2.3	2.6	3.1	3.2	3.6	3.2
		39	10.2	1.1	2.1	2.7	3.5	3.5	3.2	3.5
	Total		46.4	4.9	8.2	10.8	13.5	14.2	1466	14.8
	%			10.8	17.7	23.3	29.	30.6	31.5	31.9
7	20	52	12.5	1.1	2.0	3.1	4.1	4.8	5.0	5.0
		48	12.0	0.9	2.1	3.0	3.1	3.5	3.2	3.2
		42	11.2	1.1	1.5	2.8	4.0	3.9	4.0	4.0
		42	11.8	1.4	2.4	3.1	3.8	4.0	3.9	4.0
	Total		47.5	4.5	8.0	12.0	15.0	16.2	16.1	16.2
	%			9.5	16.9	25.3	31.6	34.	34.	34.1
8	25		2 died without regeneration							
		48	12.3	0.5	0.5	dead				
		49	12.1	0.6	dead					
	Total		24.4	1.1	0.5					
	%			4.5	4.0					
9	30	died without regeneration.								
10	0	50	11.6	1.1	2.1	2.8	3.0	3.5	4.1	4.1
		45	12.5	1.3	2.0	3.1	3.8	5.0	5.0	5.0
		41	12.4	1.3	2.0	3.0	4.5	4.1	4.7	4.7
		42	11.0	1.6	2.5	3.0	3.8	4.1	4.0	4.0
	Total		47.5	5.3	8.6	11.9	15.1	16.7	17.7	17.8
	%			11.1	18.1	25.	32.	35.	37.3	37.4

TABLE 21 EXPERIMENT 14

A. Normal Carbon dioxid production in distilled water.

Date	Dec.17.	Dec.18.	Dec.19.	Dec.21.	Dec.21.	Total	Av. per day
Tadpole	CO_2 produced as 0.01N H_2CO_3						
1.	6.4	7.4	7.2	6.9	6.5	34.4	6.9
2.	5.5	6.1	5.8	5.5	5.5	28.45	5.69
3.	6.6	7.3	6.85	6.4	6.6	32.8	6.66
4.	5.7	5.7	7.35	6.6	5.5	30.85	6.17

B. Carbon dioxid production in acid - HCl.

Tadpole	1	2	3	4
N/100 HCl in 200 cc of water	0	5	10	15
PH	7	5.4	3.8	3.2
Date	CO_2 as 0.01N H_2CO_3			
Dec. 22	5.9	4.4	5.4	2.4
23	5.2	4.3	2.4	2.0
24	7.1	7.5	6.0	4.3
25 & 26	11.9	10.1	5.4	5.0
27	4.8	4.8	3.8	2.2
28	7.6	6.5	5.9	2.5
29	7.0	7.0	3.7	3.3
30	7.0	6.3	3.2	1.8
31	4.55	4.4	2.15	1.5
Jan. 1	7.4	6.7	3.7	3.0
2	6.0	4.1	3.05	1.7
3	5.3	6.2	1.7	1.6
4	5.3	4.7	2.5	1.3
5	8.2	3.6	3.0	1.6
6	5.8	5.0	2.6	0.9
7	6.1	6.2	2.9	1.3
8	6.0	6.1	4.4	1.5
9	6.0	5.2	2.3	1.5
*10	6.5	5.2	4.7	1.8
11	5.8	5.5	4.5	1.5
12	6.6	5.9	4.7	3.3
13	6.1	5.6	4.9	2.75
14	6.7	5.1	2.1	2.4
15	6.3	4.6	2.0	0.9
16	7.6	5.1	4.8	3.0
17	10.6	3.9	3.7	3.1
18&19	10.8	6.5	7.9	6.1
20	7.5	4.4	4.9	3.3
21	8.4	3.4	5.8	2.6
22&23	10.5	7.3	5.8	5.3
24	7.0	5.7	6.0	3.5
25	6.8	4.3	3.9	3.0
26	8.2	6.9	5.6	1.5
27	6.0	4.4	3.7	2.2
28	6.0	5.8	3.8	1.5

Tadpole	1	2	3	4
Date				
Jan. 30&31	12.5	9.2	6.7	6.7
Feb. 1&2	10.0	5.2	4.6	4.3
3&4	11.9	8.0	7.2	3.8
5	6.3	4.2	5.5	2.3
6	6.8	2.3	4.5	dead
7	5.4	3.9	2.6	
8	6.7	5.2	4.5	
9	6.5	6.2	1.5	
10	5.8	3.8	3.6	
11	5.1	2.5	2.2	
12	6.3	5.3	dead	
13	6.5	5.6		
14		dead		

Summary

Time in days	53	53	51	45
Total CO_2	333.9	254.1	185.8	105.35
Av, CO_2 per day	6.3	4.8	3.64	2.34
% normal CO_2	91.3	84.3	55.5	37.9

* Temperature raised from 13 to 20 degrees C.

TABLE 23 EXPERIMENT 14

Regeneration in Acid - HCl

Tadpole	1	2	3	4
Length mm.	75	71	72.5	68
Length of tail	46	47	43	43
Length removed	13.2	12.5	12.	12.1
N/100 HCl in 200 cc water	0.	5	10	15

Date	Amount regenerated							
	length in mm.	%	length in mm.	%	length in mm.	%	length in mm.	%
Jan.5	1.0	.7.6	0.6	.4.7	0.5	.4.2	--	
13	1.7	12.9	1.3	10.4	0.8	6.6	0.4	3.3
20	2.6	19.7	1.9	15.2	1.8	15.	0.5	4.1
25	3.0	23.0	2.3	18.4	23	19.	0.8	6.6
30								
30	3.5	26.3	2.9	23.	2.6	21.8	0.8	6.6
Feb.3	4.0	30.	3.2	25.3	2.9	24.	1.0	8.3
7	4.5	34.	3.2	25.6	3.0	25.	dead	
12	4.5	34.	3.2	25.6	3.0	25		
14	4.5	34.	dead		dead			

TABLE 23 EXPERIMENT 15

Relation of Size and Carbon dioxid Production

Tadpole	1		2	
Wt.gms.	1.6		0.86	
Date	CO_2	CO_2 per gram	CO_2	CO_2 per gram
9/25/17	2.35	1.47	1.05	1.22
9/26/17	1.7	1.06	1.05	1.22
9/27/17	2.3	1.44	1.3	1.55
TOtal	6.35	3.97	3.4	3.99

TABLE 24 EXPERIMENT 16

Relation of Size and Carbon dioxid Production.

No.	Tad.	Length in mm.			Width	Width	Depth	Area	Weight
	no.	total	of tail	of body	body	tail		computed	
1	1	73	48	25	15	11	11.5	1766	3.7
	2	76	50	26	15	11	11.	1879	3.6
	3	77	50	27	16.2	12	11	1918	4.7
					TOTAL			5563	12.
2	1	62	39	23	13.2	9	10.3	1377	2.2
	2	63	41	22	13	10	10.2	1339.7	2.5
	3	66	42	24	13.3	10	10.1	1424.5	3.0
					TOTAL			4041.2	7.7
3	1	56	37	19	10	7	7.7	883.2	1.2
	2	53	33	20	11.6	8	7.7	874	1.3
	3	49	31	18	9.5	8.5	7.0	744.7	1.0
					TOTAL			2501.9	3.5

Carbon dioxid Production in Distilled Water.*

No. Date	1	2	3
1/22/18	54.1	40.1	19.8
1/23	46/3	30.6	13.9
1/24	54.8	36.7	16.5
1/25	71.6	45.1	16.9
1/26	54	32	20.5
1/27	47.6	31.1	17.5
Total	328.4	215.6	105.1
CO_2 per day	54.73	39.96	17.51
per day gram	4.56	4.66	5.0

Relation of Area, Weight and CO_2 production.
No.1 taken as 100.

Area	100	74.1	44.6
Weight	100	64.17	29.17
CO_2 produced	100	65.3	32.

* CO_2 given as cc.0.01N H_2CO_3

TABLE 25 EXPERIMENT 17

Relation of Size and Carbon dioxid Production.

No.	Tad. no.	Length in mm. total	of tail	of body	Width body	Width of tail	Depth c.	sq. mm. Area computed	Weight gms.
1	1	72	44	28	14.5	13.5	12	1959	4.1
	2	71	44	27	15.	11.	12	1566	3.5
	3	70	43	27	14	12	11	1736	3.6
				TOTAL				5261	11.2
2	1	60	38	22	12	8	9	1094	2.0
	2	54	32	22	12	9	9	1041	1.7
	3	60	40	20	11	8	7	984	1.4
				TOTAL				3119	5.1
3	1	49	31	18	9	7.5	6.5	768	1.0
	2	52	34	18	9	8	6.5	839	1.0
				TOTAL				1607	2.0

Carbon dioxid Production in Distilled Water

No.	1	2	3
Date			
2/4/18	43.1	26.3	13.7
2/5	42.8	26.5	9.
2/6	50.6	21.6	9.3
2/7	55.3	29.0	13.1
2/8	38.5	15.2	5.5
2/9	31.7	19.0	8.7
2/10	31.9	15.5	5.4
2/11	30.4	16.7	7.4
Total CO_2	324.3	169.8	72.1
CO_2 per day	40.5	21.2	9.
CO_2 per gram day	3.61	4.16	4.5

20 cc.HCl per 400cc. Water or 0.005N HCl

	1	2	3
2/12	24.8	8.0	3.7
2/13	27.0	16.2	6.0
2/14	20.9	7.5	3.8
2/15	22.0	12.3	dead
2/16	26.6	dead	
2/17	29.8(1 dead)	[illegible]	
T Total CO_2	151.1	44.0	13.5
CO_2 per day	25.2	11.0	4.5
CO_2 per gm.day	2.25	2.16	2.25
Decrease from normal CO_2 per da.	15.3	10.2	4.5

Ratio of Wt. Area and Decrease in CO_2 taking no.1 as 100

	1	2	3
Weight	100	45.5	17.85
Area	100	59.3	30.5
CO_2 decrease	100	66.6	30.

TABLE 26 EXPERIMENT 18

Effect of Size on CO_2 production and Regeneration

No.	No. of Tads	Av. length m.m.	Total wt.	Total area sq. mm.	CO_2 Produced 3/9	3/10	3/11	3/12	3/13	3/14
1A	1	79	3.6	1852	28.3	24.3	19.2	18.4	21.8	19.8
1B	3	54	3.4	3092	24.9	23.6	16,2	16.3	16.9	18.4
1C	6	44	3.4	3924	26.7	22.3	19.1	17.1	17.2	19.1
2A	1	82	3.2	2104	22.2	20.2	20.5	12.7	17.0	15.2
2B	3	56	3.2	3076	22.7	20.7	18.5	17.5	16.3	17.0
2C	7	40	3.4	4164	26.8	22.3	19.3	19.7	19.6	18.6
3A	1	75	2.7	1932	26.2	25.8	19.0	19.9	20.3	14.5
3B	2	61	2.7	2369	19.3	19.1	16.0	15.9	15.7	18.3
3C	6	26	2.7	3547	16.1	18.0	15.4	12.5	14.7	14.7
4A	1	76	2.7	1800	18.6	17.5	16.1	14.5	14.5	11.8
4B	2	55	2.3	1913	17.6	14.7	12.1	11.3	12.0	12.7
4C	6	39	2.7	3323	17.9	20.9	14.9	14.0	14.7	13.5

The same tadpoles with 20c.c. 0.01 N KOH, $Ca(OH)_2$ and H_2SO_4 made up tp 400 cc.

SUMMARY

	3/15	3/16	3/17	3/18	CO2 per day normal	CO_2 per day in expt.	% of normal CO2
Control							
1A	12.2	10.5	13.6	14.8	21.97	12.8	58.4
1B	14.3	13.7	14.5	14.7	19.4	14.3	74.
1C	14.2	13.8	14.3	16.3	20.25	14.65	72.
KOH							
2A	22.5	19.0	19.0	20.8	17.97	15.8	88.
2B	24.9	22.3	21.4	21.6	18.8	22.55	107.
2C	25.6	dead(5)			21.05	25.6	121.
$Ca(OH)_2$							
3A	21.6	19.0	16.1	13.5	20.93	17.55	84.
3B	26.8	21.6	17.5	15.6	17.4	20.4	117.
3C	24.3	2 dead			15.23	24.3	159.
H_2SO_4							
4A	10.3	7.4	12.8	5.0	15.5	8.87	56.5
4B	12.0	9.1		9.7	13.23	10.3	78.
4C	11.4	5.6		9.7	15.98	8.9	56.

SUMMARY Continued

No.	CO_2 per day per Gram normal	in expt.	No.	CO_2 per gram per day normal	in expt.
1A	5.55	3.55	3A	7.7	6.5
1B	5.7	4.2	3B	6.45	7.55
1C	6.0	4.3	3C	5.65	9.0
2A	5.63	4.95	4A	5.65	3.28
2B	5.9	7.05	4B	6.0	4.7
2C	6.2	7.55	4C	5.9	3.3

TABLE 27 EXPERIMENT Continued

Effect of Size upon CO_2 Production and Regeneration

The Surviving Tadpoles of the previous experiment were operated and allowed to undergo regeneration in the same solutions as those in which the CO_2 production had been determined.

No.	No. of Tads.	Length removed 3/18/18	Length Regenerated in mm. 3/23	3/26	3/30	4/3	4/9	4/13	4/16
Controls									
1A	1	16	1.1	2.1	4.4	4.5	6.0	6.5	6.5
	%		6.9	13.1	27.	28.	37.	41.	41.
1B	2	24.3	1.5	4.9	7.4	8.4	9.7	9.7	9.7
	%		6.2	20.	30.	35.	40.	40.	40.
1C	6	62.4	6.6	14.7	22.1	26.0	29.6	30.2	29.7
	%		11.5	23.5	35.6	41.5	47.5	51.	48.
KOH									
2A	1	16.1	1.0	2.0	3.6	5.5	6.8	6.9	7.0
	%		6.2	12.4	22.	34.	42.	43.	43.
2B	2	23.9	1.5	3.9	7.0	8.1	10.0	10.1	10.1
	%		6.3	16.3	29.	34.	42.	42.5	42.5
2C	2	20.6	1.9	3.7	5.6	6.1	6.4	6.5	dead
	%		9.2	18.	27.	29.6	31.	31.6	
$Ca(OH)_2$									
3A	1	16.6	1.1	3.0	5.0	6.1	7.0	7.0	7.0
	%		6.6	18.	30.	36.8	42.	42.	42.
3B	2	24.6	1.7	3.5	6.8	7.8	10.6	10. 5	10.8
	%		6.9	14.	28.	32.	43.	43.	44.
3C	4	40.1	4.4	8.0	12.6	14.4	15.1	15.1	15.1
	%		11.	20.	35.	36.	38.	38.	38.
H_2SO_4									
4A	1	16.2	1.0	2.7	5.0	7.0	7.0	7.0	7.0
	%		6.2	16.7	31.	43.	43.	43.	43.
4B	2	23.9	2.2	4.3	6.3	7.6	8.0	7.8	7.8
	%		9.2	18.	26.	32.	33.4	32.5	32.5
4C	6	59.7	6.9	13.4	18.9	20.4	21.2	dead	
	%		11.4	22.4	32.	33.	35.4		

TABLE 28 EXPERIMENT 19

Effect of Size on Regeneration in Acids - HCl

cc.HCl per l.	No.of Tads.	Length av.	Length removed 4/7/18	Length Regenerated in mm. 4/15	4/17	4/19	4/22	4/24	4/27	4/30
0	3	54	42.9	5.9	9.5	10.7	14.0	15.2	16.6	18.0
%				13.8	22.	25.	33.	35.5	39.	42.
	3	43	36.4	5.9	7.9	9.8	12.2	13.2	14.4	15.0
%				16.4	21.7	27.	33.5	36.	39.6	41.
	2	28	16.4	3.8	4.8	6.1	6.7	7.5	8.1	8.1
%				23.	29.	37.	41.	46.	49.4	49.4
0'	3	51	42.	6.9	9.8	11.2	14.1	15.2	16.1	16.2
%				16.4	23.3	26.7	33.6	36.	38.	38.6
	3	41	34.1	6.2	9.0	11.7	12.8	14.8	15.5	16.0
%				18.2	26.4	34.2	37.6	43.5	45.5	47.
	4	32	40.5	9.1	12.3	13.8	16.0	16.6	17.9	17.8
%				22.4	30.4	34.	39.5	41.	44.	44.
Average % of Regeneration of the two above Controls										
	6	52.5		15.1	22.65	25.85	33.3	35.75	38.5	40.3
	6	42		17.3	24.05	30.6	35.55	39.75	42.5	44.15
	6	30		22.7	29.7	35.5	40.25	43.5	46.7	46.7
30	3	55	45.5	5.9	9.0	10.3	12.4	13.4	14.5	15.3
%				13.	19.8	22.6	27.3	29.5	32.	33.6
	3	41	33.4	5.8	9.5	10.5	11.8	13.0	14.2	14.2
%				17.3	28.4	31.4	35.	39.	42.4	42.5
	4	33	38.5	7.3	10.1	11.8	14.1	14.8	15.9	16.3
%				19.5	26.2	30.6	36.6	37.5	41.5	42.5
40	2	51	29.	4.0	5.2	7.6	9.0	9.6	9.9	10.2
%				13.8	18.	26.2	31.	33.	34.	35.
	3	42	36.	6.4	9.0	10.6	12.4	13.1	13.6	14.1
%				17.8	25.	29.4	34.5	36.4	38.	39.
	4	33	37.7	7.3	11.6	12.7	13.2	15.0	15.1	15.2
%				19.4	31.	34.	35.	40.	40.	40.4
50	3	56	44.2	6.1	8.5	10.3	11.9	12.7	13.7	13.8
%				13.8	19.2	23.0	27.0	29.0	31.0	31.2
	3	41	34.9	6.0	7.2	8.6	10.3	11.8	11.7	dead
%				17.2	20.6	24.6	29.5	34.	34.	33
	4	33	35.5	7.2	9.5	11.9	13.4	13.6	13.8	dead
%				20.3	27	33.5	38	38.2	39	

% of Regeneration Compared to the Controls
Controls = 100%

cc.HCl per l.	Length av.	4/15	4/17	4/19	4/22	4/24	4/27	4/30
30	55	86.	88.	88.	82.	83.	83.	83.5
	41	100	118.	102.	98.5	98.	99.6	96.5
	32	86.	89.	86.4	90.	86.	89.	89.5
40	51	91.5	79.5	101.	93.	92.	88.	87.
	42	103.	104.	96.	97.	91.5	89.	88.5
	33	85.5	104.	96.	87.	92.	86.	86.5
50	56	91.5	84.5	89.	81.	81.5	81.	77.5
	41	99.5	93.5	80.5	83.	86.	80.	75.
	32	90.	91.	94.	95.	90.	83.5	

TABLE 29 EXPERIMENT 20

Regeneration in low Oxygen

Jar	1	2	3	4	5	6	7
cc.O_2 per liter							
maximum	0	1.04	2.5	2.9	3.68	4.35	5.22
minimum	0	0.133	1.0	2.0	2.54	2.93	4.1
average	0	0.54	1.6	2.48	3.03	3.8	4.7
Tadpole							
length	77	72	73	77	70	72	70
removed	11.0	13.7	12.0	12.5	12.8	13.0	11.5
Regenerated							
Date begun 12/18/17							
12/29	dead	0.6	1.0	1.0	1.0	1.2	1.2
%		4.4	8.4	8.	7.8	9.3	10.5
1/6/18		1.5	2.1	1.4	2.2	2.0	2.1
%		11.0	17.5	11.2	17.0	15.4	18.0
1/10		2.0	2.2	2.0	2.4	2.0	2.5
%		14.6	18.0	16.0	19.	15.4	22.
1/15		2.1	2.5	2.2	2.8	2.1	3.0
%		15.	21.	17.5	22.	16.	26.
1/20		2.5	2/9	2.6	3.4	2.8	3.2
%		18.3	24.	20.8	26.	22.	28.
1/23		dead	3.1	2.5	3.0	2.8	3.2
%			25.	20.	23.	21.5	28.
1/28			3.1	2.5	3.0	dead	3.2
%			25.	20.	23.		28.

TABLE 30 EXPERIMENT 20

Regeneration in Low Oxygen

Jar		1	2	3	4	5	6	7
$cc.O_2$ per liter								
maximum		0	1.04	2.5	2.9	3.68	4.35	5.22
minimum		0	0.133	1.0	2.0	2.54	2.93	4.1
average		0	0.54	1.6	2.48	3.03	3.8	4.7
Tadpole length		52	53	53	58	59	52	50
removed		10	10.1	10.8	10	10	9.5	9.8
Regenerated date begun		12/18/17						
12/29		0.5	dead	1.4	0.5	0.4	0.9	1.6
	%	5.		13.	5.	4.	9.	16.3
1/6		1.5		2.8	1.0	2.1	1.2	2.7
	%	15.		26.	10.	21.	12.7	28.
1/10		2.0		3.1	1.5	dead	1.4	3.0
	%	20.		28.7	15.		14.8	30.
1/15		2.1		3.2	1.9		1.5	3.1
	%	21.		29.6	19.		15.8	32.
1/20		2.5		3.2	2.1		2.0	3.1
	%	25.		29.6	21.		21.	32.
1/23		2.5		3.5	dead		2.2	3.2
	%	25.		32.			23.	33.
1/28		2.5		3.5(dead)			2.2	3.2
	%	25.		32.			23.	33.
Tadpole length		37	35	34	36	34	36	34
removed		8	7.5	7	7.2	7.5	8	8.1
Regenerated 12/29		dead	0.5	0.8	dead	dead	dead	2.0
	%		6.6	14.3				25.
1/6			dead	dead				3.1
	%							38.
1/10								3.3
	%							41.
1/15								3.6
	%							44.
1/20								3.8
	%							47.
1/23								3.8
	%							47.

TABLE 31 EXPERIMENT 31

Regeneration in Low Oxygen Water.

Jar no.	1	2	3	4	5	6	7
cc.O_2 per liter							
maximum	trace	1.14	1.8	2.9	3.6	4.68	5.0
minimum	0	0.37	0.77	1.1	2.4	2.7	3.3
average	0+	0.7	1.2	1.9	3.17	3.68	4.1

Jar.	Length in mm.	Length removed in mm.	Length Regenerated in mm. Begun 3/8/17							
			3/19		3/22		3/28		4/3	
			mm.	%	mm.	%	mm.	%	mm.	%
1	75	17	1.6	9.3	2.8	16	5.2	30.6	5.2	30.6
	67	17.4	1.2	7.0	3.0	17.2	5.6	32.	5.6	32.
	Av.%			8.1		16.6		31.3		31.3
2	71	16.4	2.6	15.8	3.8	23.	6.4	39.	6.2	38.
	65	17	2.8	16.4	4.0	23.5	6.3	37.	6.1	36
	Av.%			16.1		23.3		38		37
3	72	17.7	2.7	15.	4.5	25.4	7.0	39.	7.5	42
	74	17	2.7	16	4.1	24	6.0	35	6.0	35
	Av %			15.5		24.7		37		38.5
4	72	18	3.1	17.	5.0	28.	7.0	39.	6.8	38.
	70	17.2	3.1	18	4.5	26	6.6	38	6.8	39.5
	Av.%			17.5		27		38.5		38.7
5	73	17.9	3.2	17.8	5.0	28.	7.1	39.6	7.3	41.
	70	17	2.8	16.5	4.8	28.	6.5	38.	6.5	38.
	Av.%			17.1		28		38.8		39.5
6	70	18	3.4	19	5.1	28.	6.6	36.6	6.0	33.4
	73	16	3.0	19	4.2	26.	7.0	44.	7.0	44.
	Av.%			19		27		40.3		38.7
7	71	17.4	4.0	28.	5.2	30.	7.3	42.	7.2	42.
	64	18.1	4.1	23.	6.0	33.	7.6	42.	7.5	42.
	Av.%			25,5		31.5		42.		42.

Nos.1 and 2 transferred to aerated water at the close of the experiment underwent no further regeneration.

TABLE 32 EXPERIMENT 22

Regeneration in Low Oxygen

Jar	1	2	3	4	5	6	7
co.CO_2 per liter							
maximum	trace	1.0	1.9	3.3	3.6	4.68	5.7
minimum	0	0.2	0.45	1.0	1.8	2.3	3.1
average	0+	0.6	1.17	1.8	2.7	3.28	4.1
Tadpoles							
length	83	79	81	85	80	82	82
removed	22.5	20.0	22.2	23.0	21.6	20.7	22.0
Regenerated							
Date - begun 3/29/18							
4/3	- -	- -	- -	- -	- -	slight but not measurable.	
4/10	4.0	3.8	4.3	5.0	5.4	5.6	6.2
%	17.5	19.	19.4	21.7	25.	27.	28.
4/15	4.5	4.8	5.5	5.9	6.1	6.1	7.6
%	20.	24.	24.8	25.6	28.	29.	34.5
4/23	4.8	5.5	6.7	6.9	6.3	7.6	8.5
%	21.3	24.8	30.	30.	29.	36.7	38.5
4/29	5.3	5.5	6.9	7.2	6.3	7.6	8.4
%	23.5	27.5	31.	31.3	29.	36.7	38.

TABLE 33 EXPERIMENT 23

Regeneration in Low Oxygen

Jar		1	2	3	4	5	6
cc.O$_2$ per liter							
maximum		.23	2.76	4.6	5.36	8.3	10.8
minimum		.13	0.5	1.9	3.8	5.0	7.1
average		.2	1.5	3.0	4.43	7.1	8.38
Tadpoles							
length		75	74	71	73	76	73
removed		17.7	17.2	20	16.3	18.1	15.3
Regenerated							
Date - begun		10/23/17					
10/26		- -	- -	-.-	- -	- -	- -
10/30		- -	0.8	0.8	0.6	0.9	- -
	%		4.6	4.0	3.6	5.0	
11/1		- -	0.9	1.1	0.9	1.3	0.7
	%		5.2	5.0	5.5	7.2	4.6
11/3		- -	1.4	dead	1.1	1.5	1.0
	%		8.1		6.8	8.3	6.6
11/7		0.3	2.4		3.1	3.7	3.0
	%	1.7	14.		19.	20.	19.
11/10		0;5	2.8		4.0	3.9	3.0
	%	2.8	16.		24.	22.	20.
11/13		0.5	3.2		4.0	4.2	3.7
	%	2.8	18.6		24.5	23.2	24.1
11/14	Boiler failed. Water allowed to become aerated.						
11/18		4.0	4.3		5.6	5.7	5.0
	%	22.6	25.		34.4	31.8	32.7
12/1		5.0	6.2		8.8	8.0	6.6
	%	28.	36.		54.	44.	43.
12/13		dead	6.2		v 8.8	8.0	dead
	%		36.		54.	44.	

TABLE 34 EXPERIMENT 24

Effect of Temperature on Regeneration

t.C.	Length in mm.	Length removed in mm.	Length Regenerated in mm. Begun 3/6/18 3/10	3/13	3/17	3/21	3/26	3/30	4/5
19	60	16	1.2	3.0	5.4	8.0	8.0	8.0	8.0
to	63	16	1.1	2.8	5.2	7.0	7.2	7.5	7.5
21	57	14	1.1	3.2	5.0	7.0	7.0	7.3	7.4
	59	14.6	1.0	2.8	4.6	5.5	5.6	6.2	6.2
	total	60.6	4.4	11.8	20.2	27.5	27.8	29.0	30.5
	%		7.3	19.4	33.3	45.5	45.9	48.	50.
14	61	15		0.6	1.1	2.4	3.2	4.4	5.7
	54	16		0.5	1.0	2.6	3.7	4.0	5.0
	62	14		0.5	1.6	2.8	3.5	4.5	5.2
	64	15		0.4	1.0	2.2	3.7	5.0	6.0
	total	60		2.0	4.7	10.0	14.1	17.9	21.9
	%			3.3	7.8	16.6	23.8	29.6	36.5
0	62	16							(T)
to	55	16			No Regeneration				
4	65	14.5							(T)
	60	15							

Date	4/16	4/20	4/23	4/29	5/4
19	8.0	8.0	8.0	8.0	8.0
to	7.5	7.8	7.8	7.8	7.8
21	7.6	7.6	7.7	7.7	7.7
	6.2	6.2	6.1	6.0	6.0
	29.3	29.6	29.6	29.5	29.5
	48.	49.	49.	49.	49.
14	6.4	6.5	6.5	6.5	6.5
	5.5	5.5	5.5	5.7	5.8
	6.0	6.0	6.0	6.0	6,0
	6.7	6.8	6.8	7.2	7.1
	24.6	24.8	24.8	25.4	25.4
	41.	41.	41.	42.3	42.3
0	3.3	4.8	5.0	6.0	6.2
to	no regeneration			dead	
4	4.1	5.2	5.3	6.2	6.5
	no regeneration			dead	
	7.4	10.0	10.3	12.2	12.7
	24.6	33.	34.4	40.	42.3

(T) = Transferred to temperature 19 to 21 degrees.

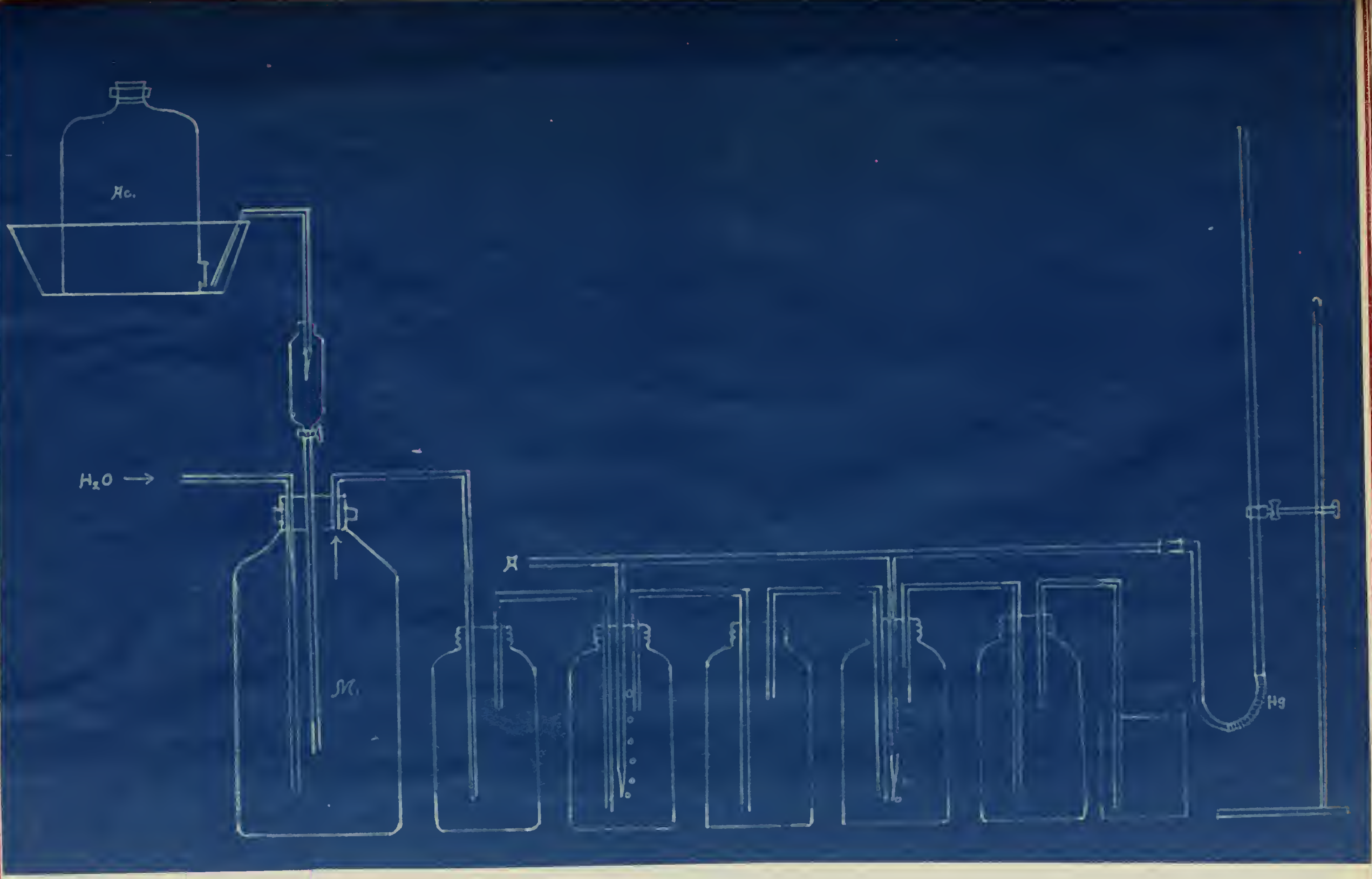

Fig.1. Apparatus for securing a series of low Oxygen concentrations.(Page b)

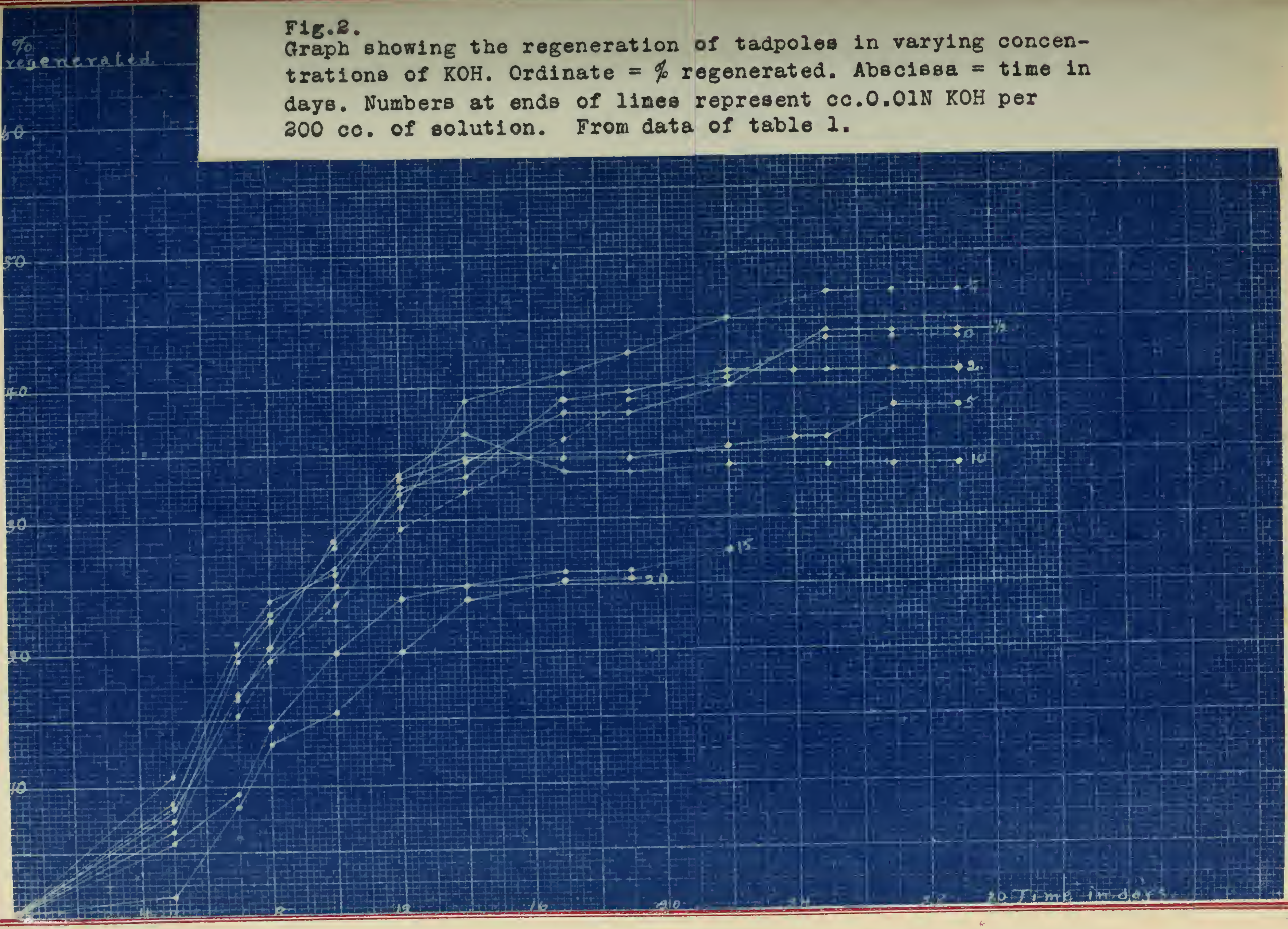

Fig.2.
Graph showing the regeneration of tadpoles in varying concentrations of KOH. Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of lines represent cc.0.01N KOH per 200 cc. of solution. From data of table 1.

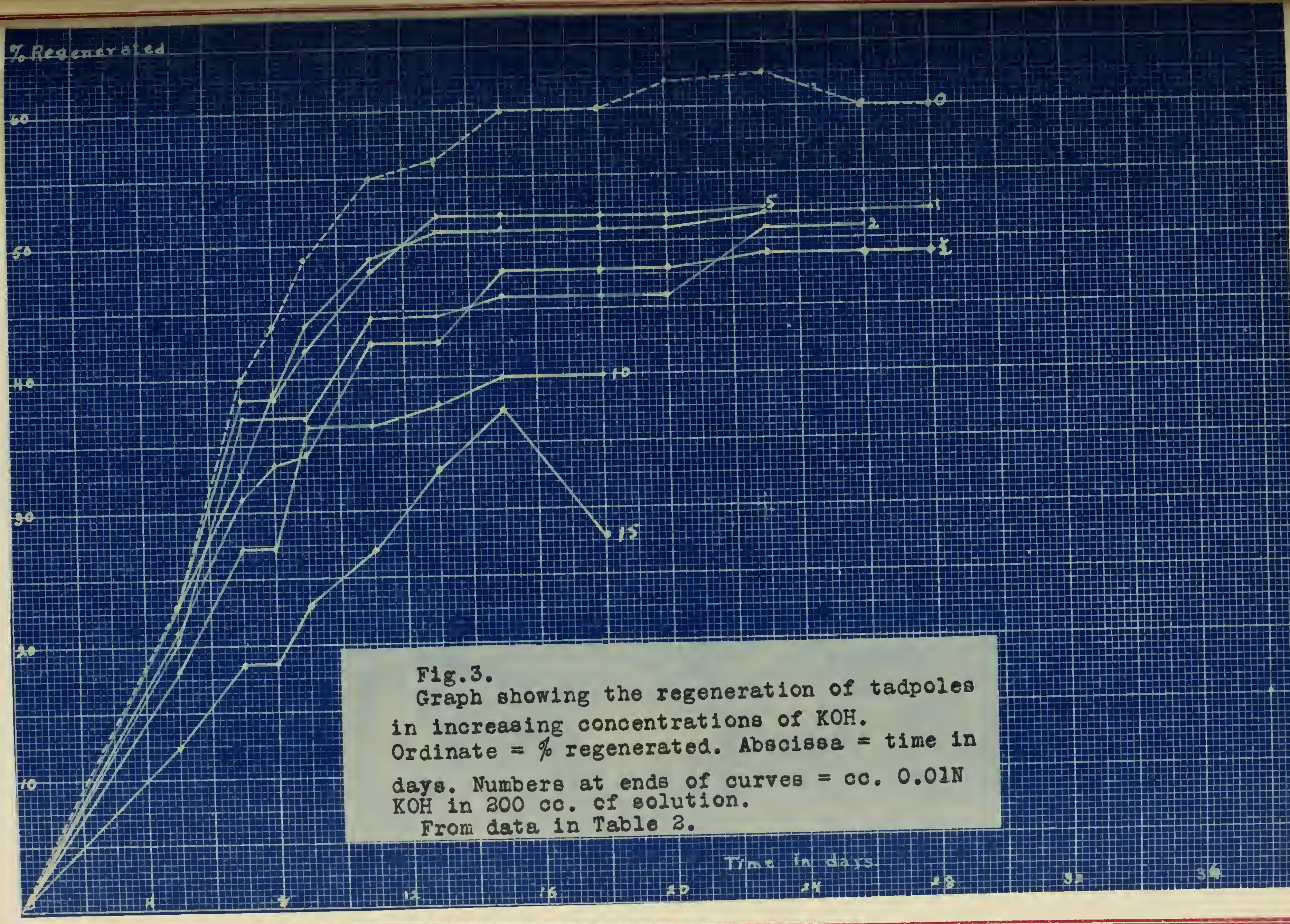

Fig.3.
Graph showing the regeneration of tadpoles in increasing concentrations of KOH. Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of curves = cc. 0.01N KOH in 200 cc. cf solution.
From data in Table 2.

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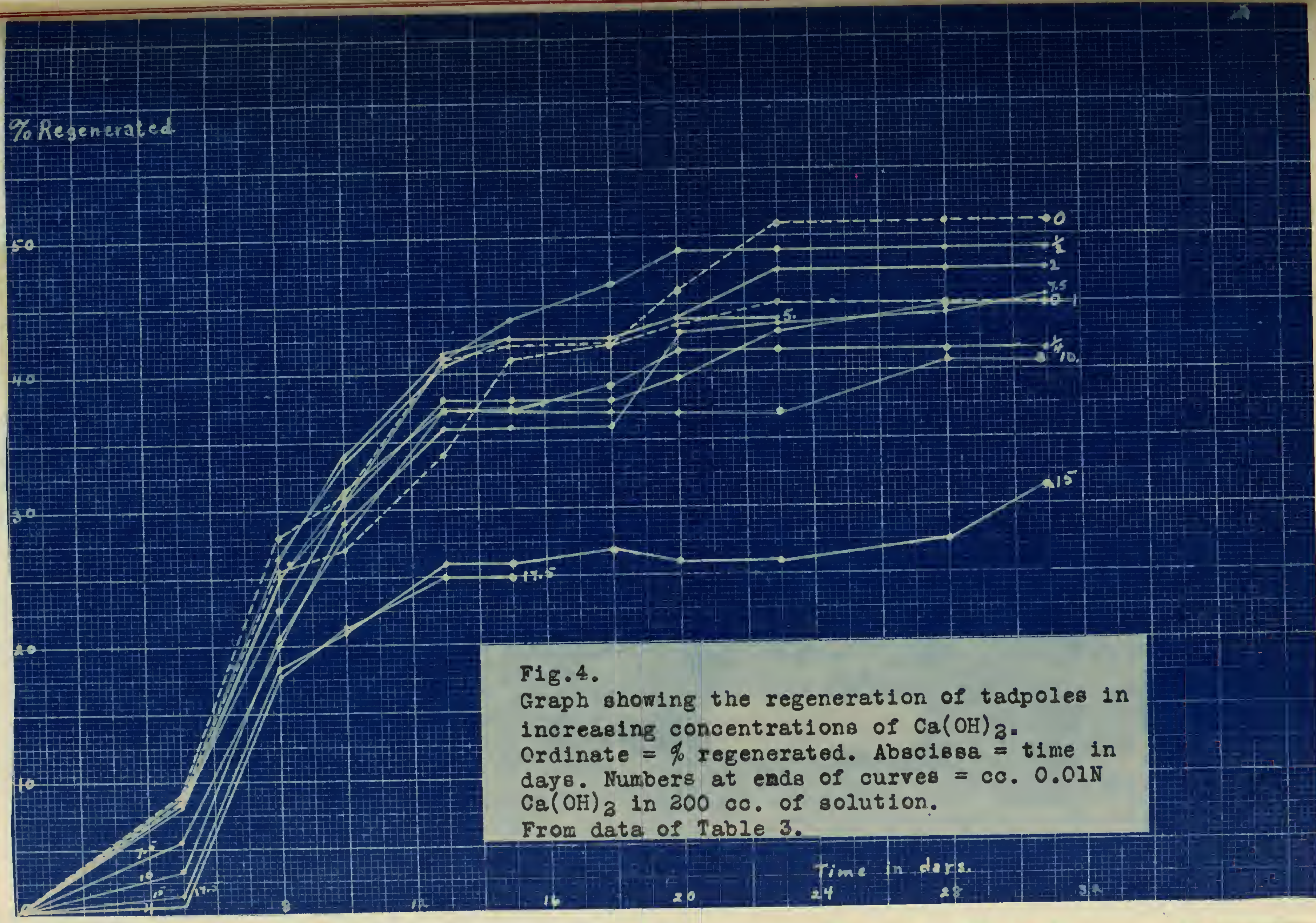


Fig.4.
Graph showing the regeneration of tadpoles in increasing concentrations of $Ca(OH)_2$.
Ordinate = % regenerated. Abscissa = time in days. Numbers at emds of curves = cc. 0.01N $Ca(OH)_2$ in 200 cc. of solution.
From data of Table 3.

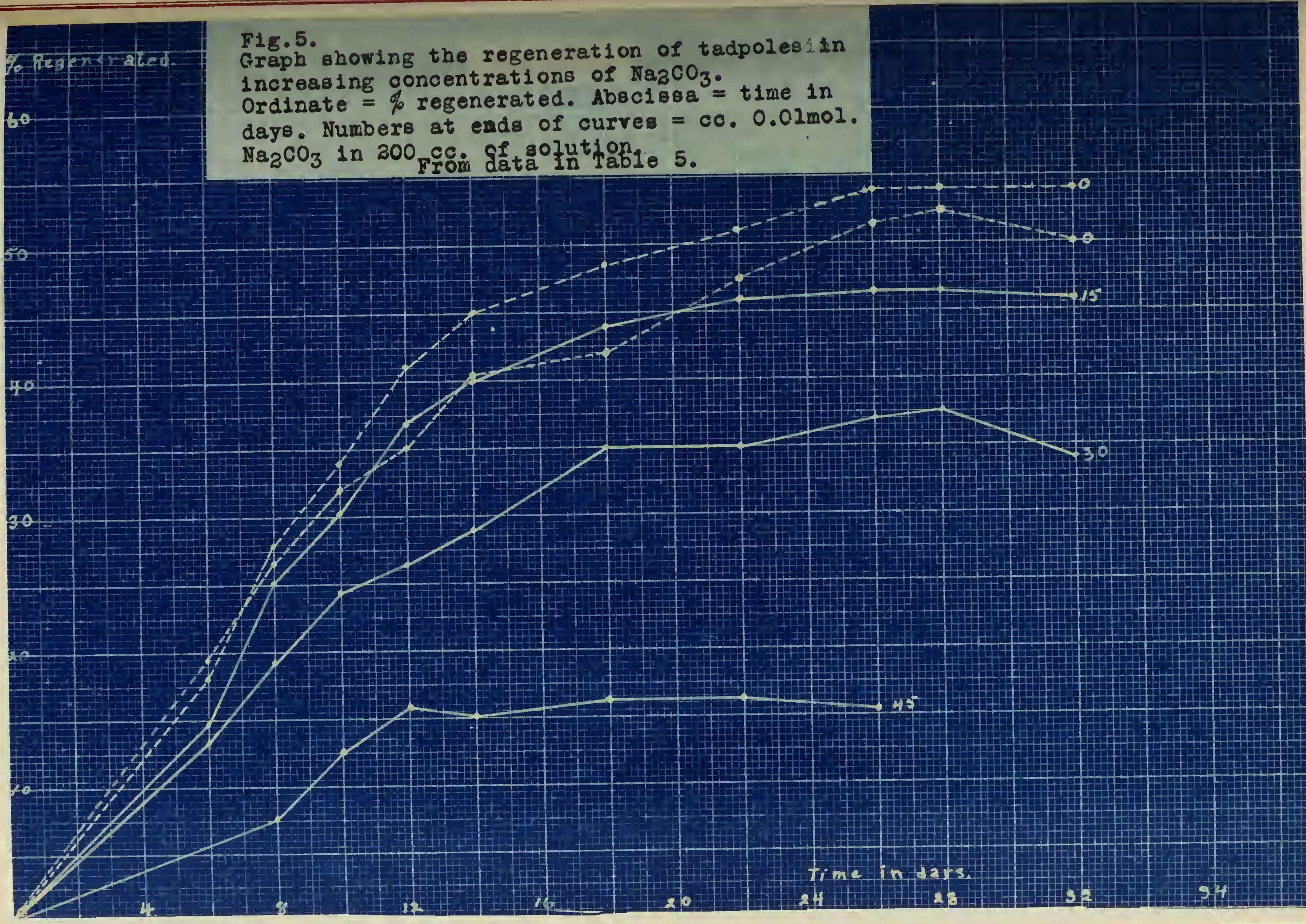


Fig.5.
Graph showing the regeneration of tadpoles in increasing concentrations of Na_2CO_3.
Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of curves = cc. 0.01mol. Na_2CO_3 in 200 cc. of solution. From data in Table 5.

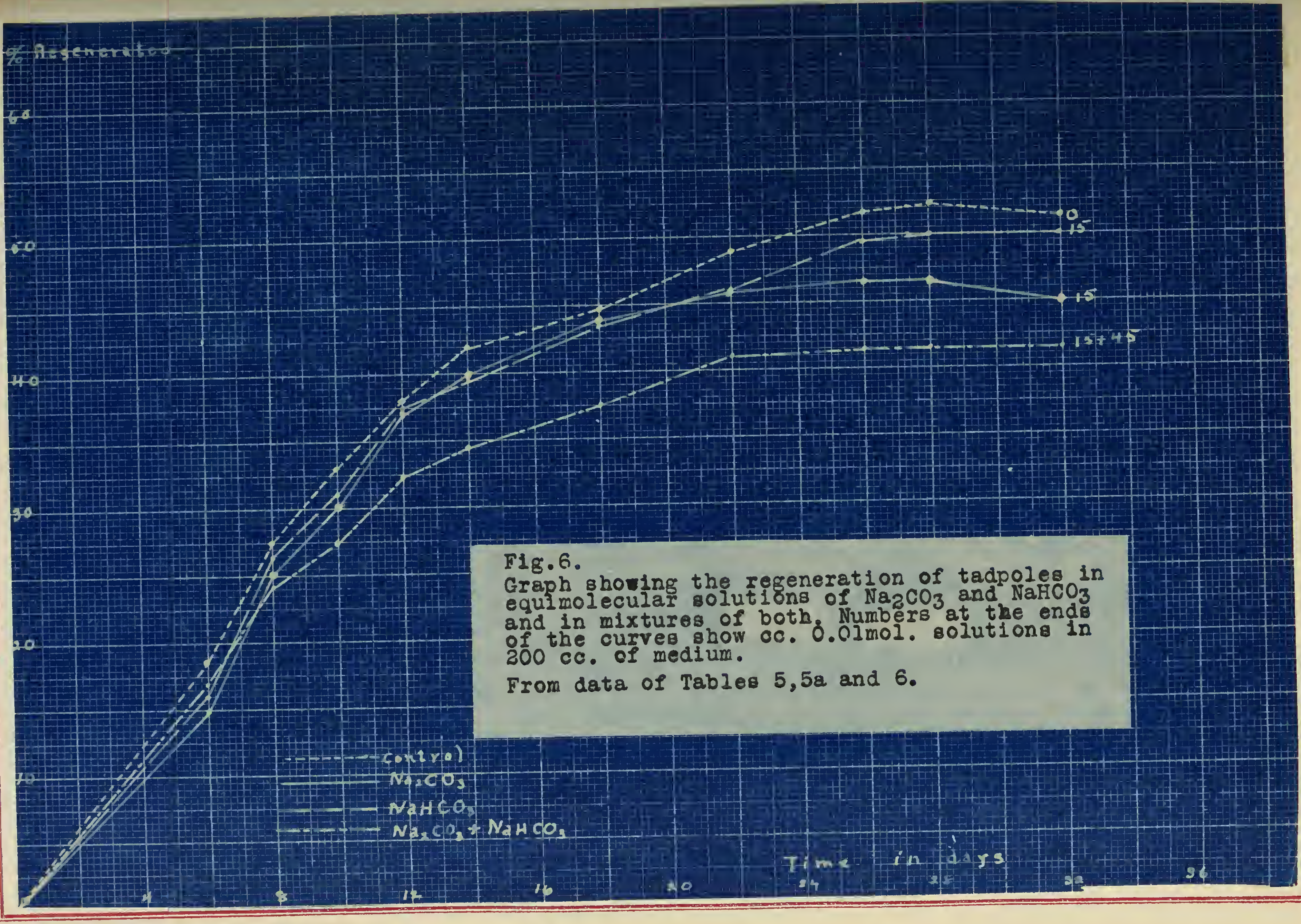


Fig.6.
Graph showing the regeneration of tadpoles in equimolecular solutions of Na_2CO_3 and $NaHCO_3$ and in mixtures of both. Numbers at the ends of the curves show cc. 0.01mol. solutions in 200 cc. of medium.

From data of Tables 5,5a and 6.

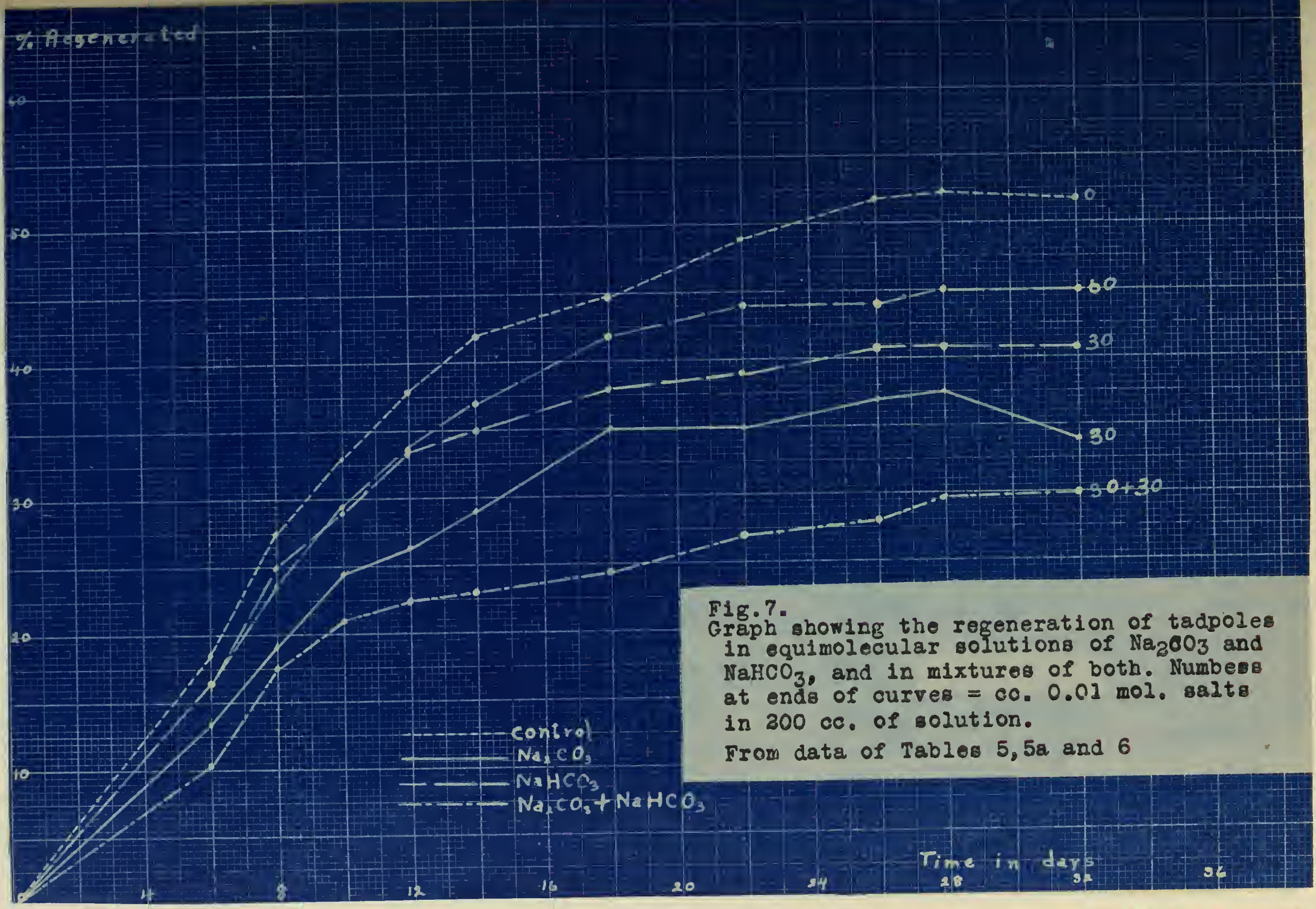


Fig. 7.
Graph showing the regeneration of tadpoles in equimolecular solutions of Na_2CO_3 and $NaHCO_3$, and in mixtures of both. Numbess at ends of curves = cc. 0.01 mol. salts in 200 cc. of solution.

From data of Tables 5, 5a and 6

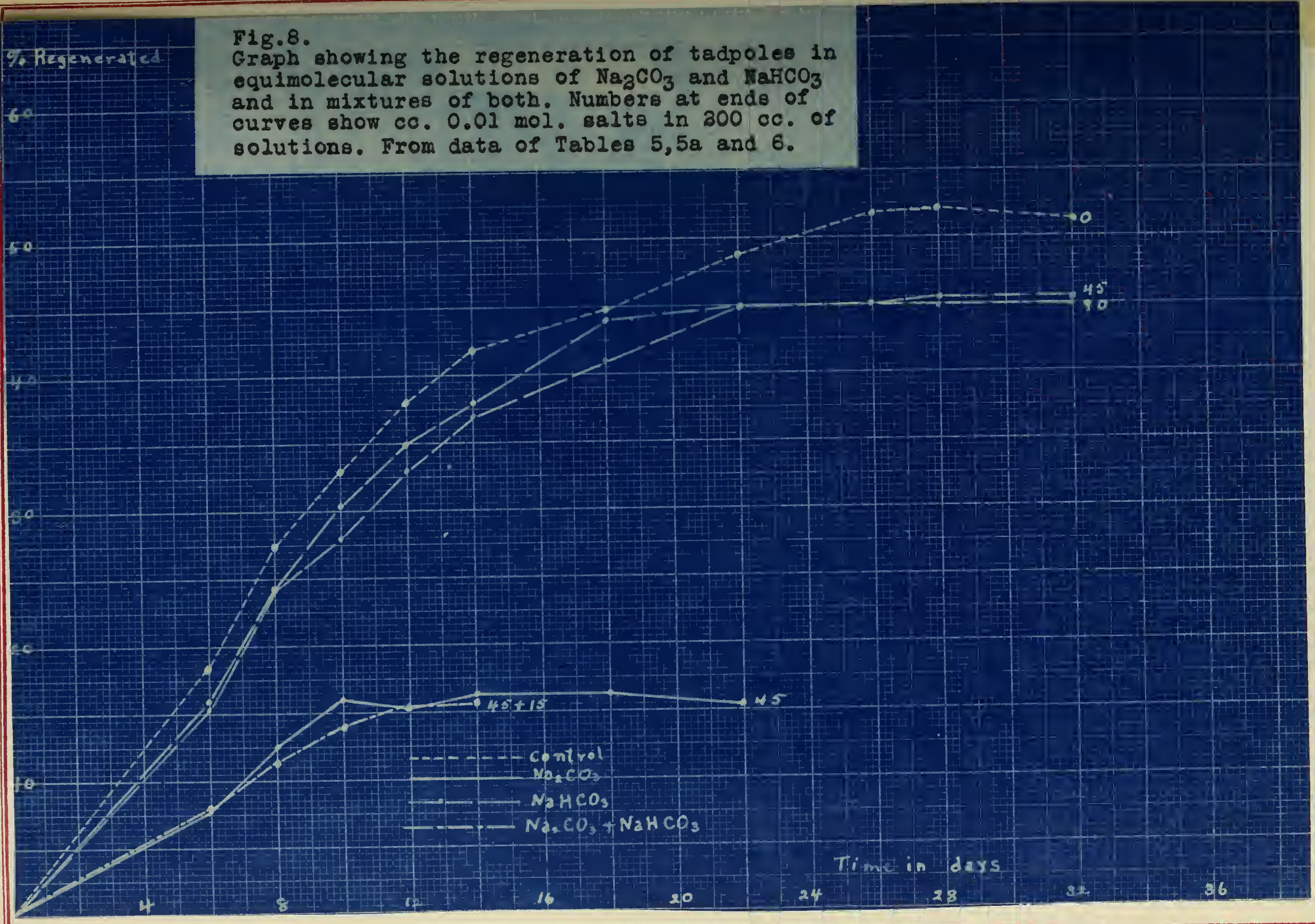


Fig.8.
Graph showing the regeneration of tadpoles in equimolecular solutions of Na_2CO_3 and $NaHCO_3$ and in mixtures of both. Numbers at ends of curves show cc. 0.01 mol. salts in 300 cc. of solutions. From data of Tables 5,5a and 6.

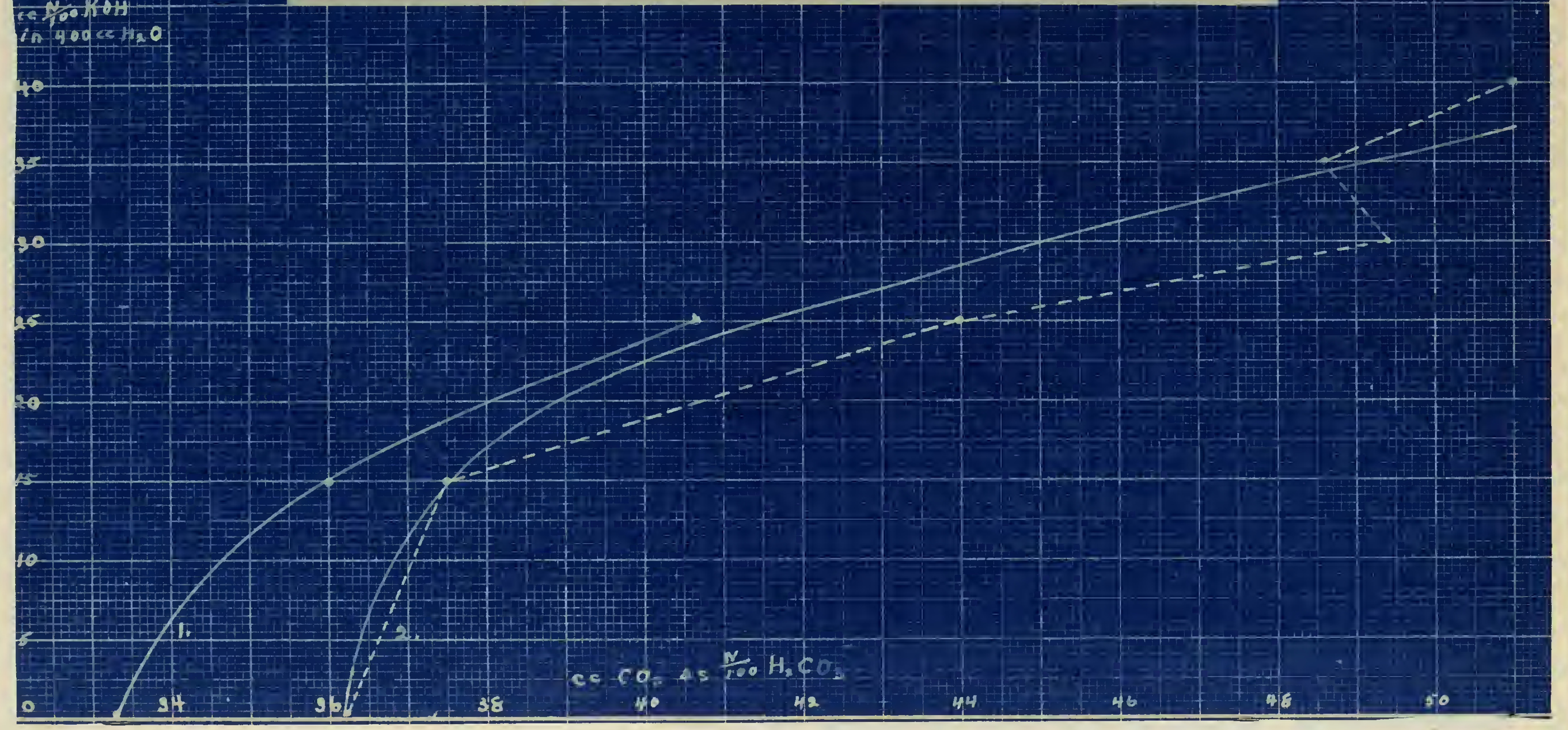


Fig.9.
Graph showing daily production of CO_2 by tadpoles in increasing concentrations of KOH. Ordinate = cc. 0.01N KOH in 400 cc. of solution. Abscissa = CO_2 produced as 0.01N H_2CO_3 Curve 1 = average of 24 days. Curve 2 = CO_2 produced in 1 day. From data of Table 7.

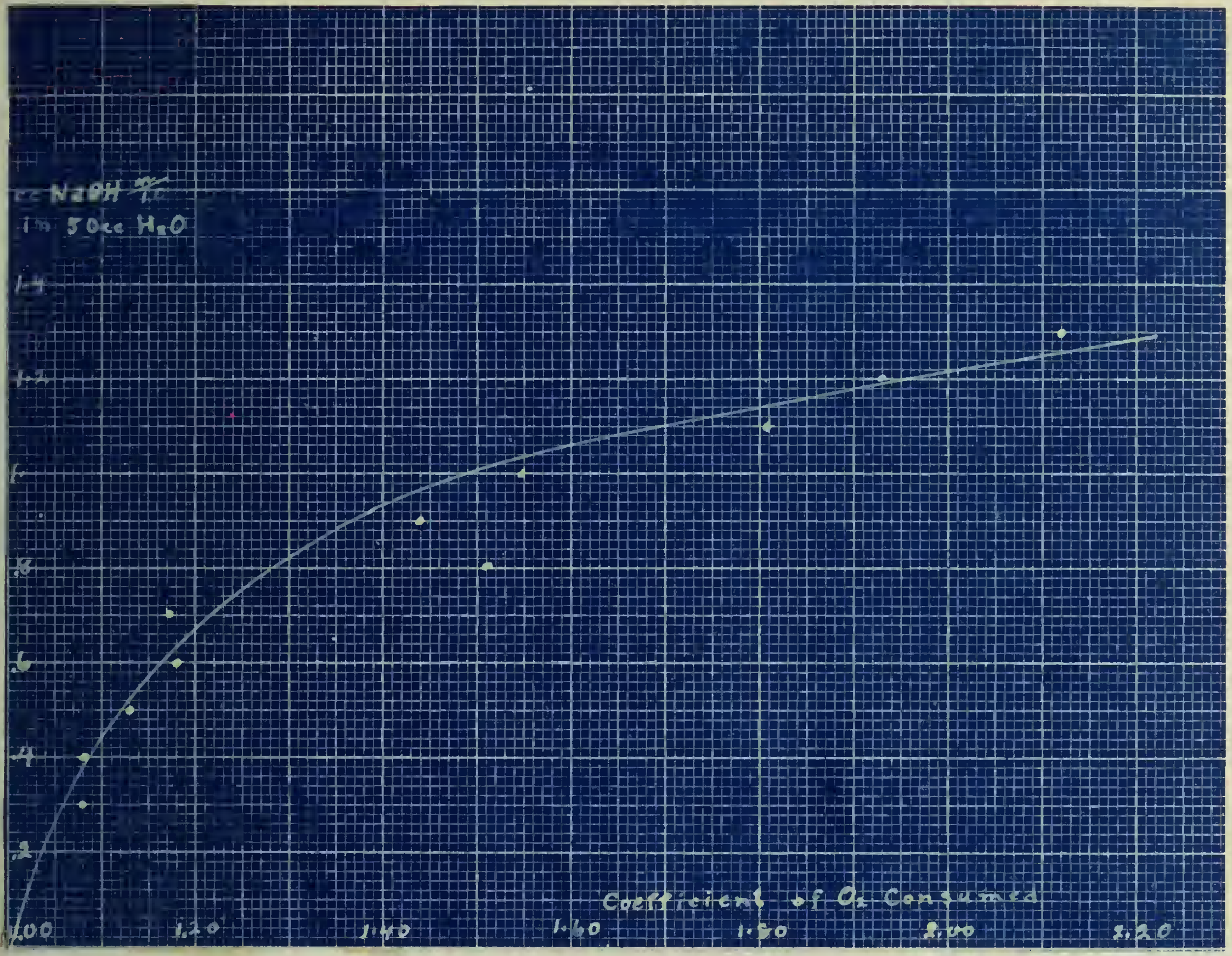


Fig.10.

Graph showing the Oxygen consumption of Fertilized eggs of Strongylocentrotus purpuratus in increasing concentrations of NaOH. Ordinate = cc. 0.1N NaOH in 50 cc. of the solution. Abscissa = coefficient of Oxygen consumed as compared to neutral controls.

Drawn from the data of Loeb and Wastneys (1913 b)

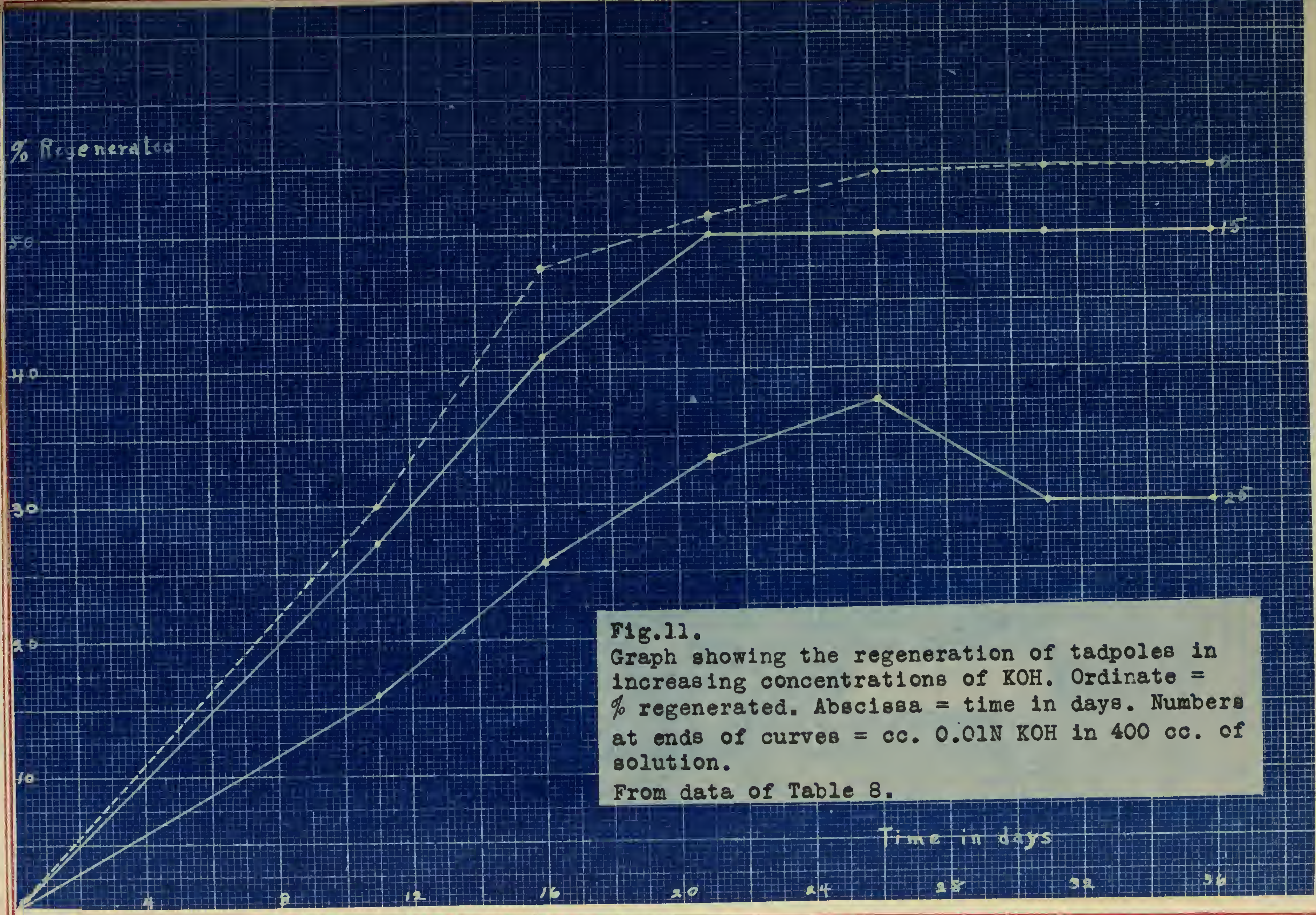
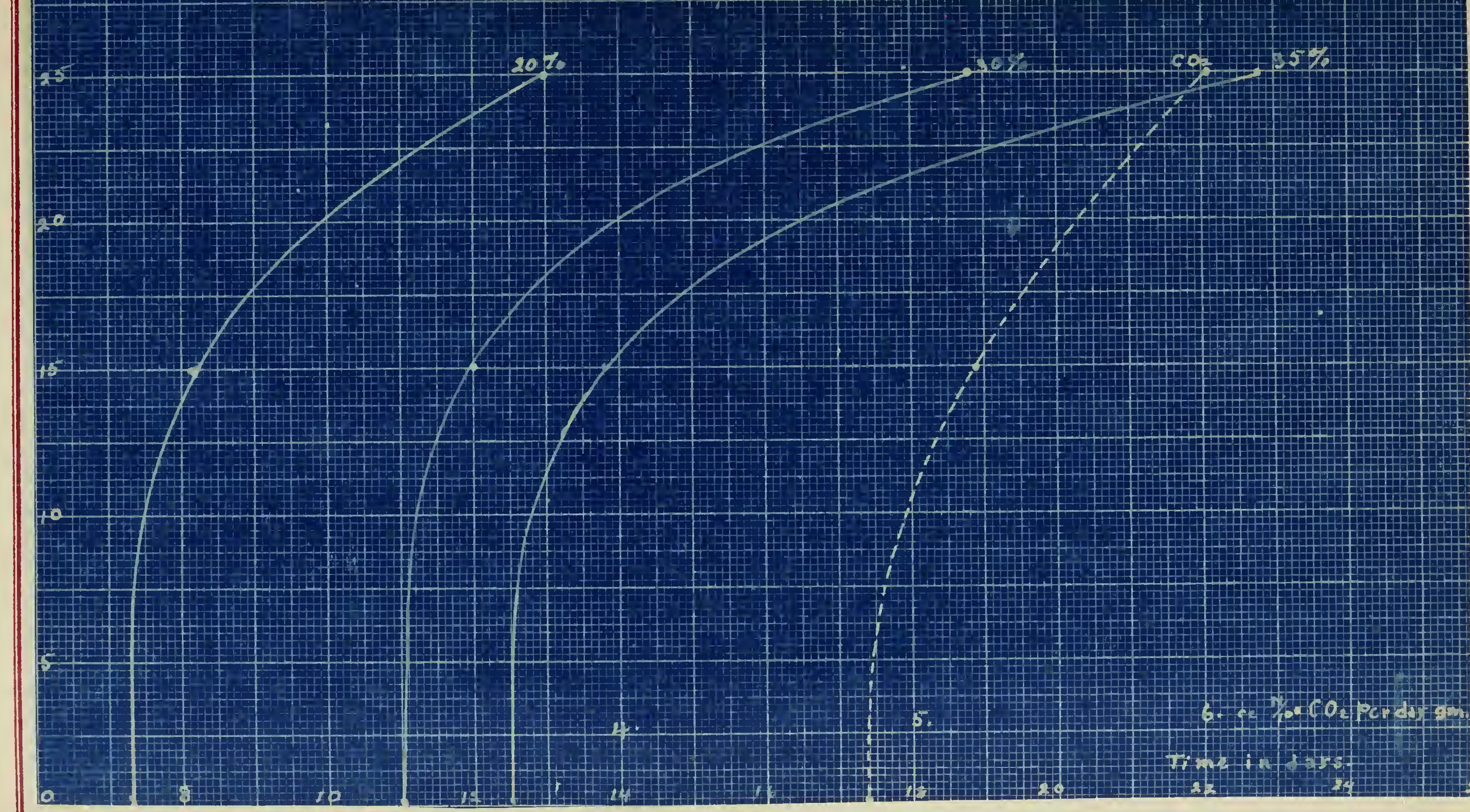


Fig.11.
Graph showing the regeneration of tadpoles in increasing concentrations of KOH. Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of curves = cc. 0.01N KOH in 400 cc. of solution.
From data of Table 8.

Fig.12.
Graph showing regeneration in increasing concentrations of KOH. Ordinate = cc. 0.01N KOH in 400 cc.of solution. Abscissa = time in days. Numbers at ends of curves = % regenerated, Dotted line = Av. daily CO_2 per gram of the same tadpoles. Ordinate as above. Abscissa = cc. CO_2 as 0.01N H_2CO_3. From data of Tables 7 and 8.



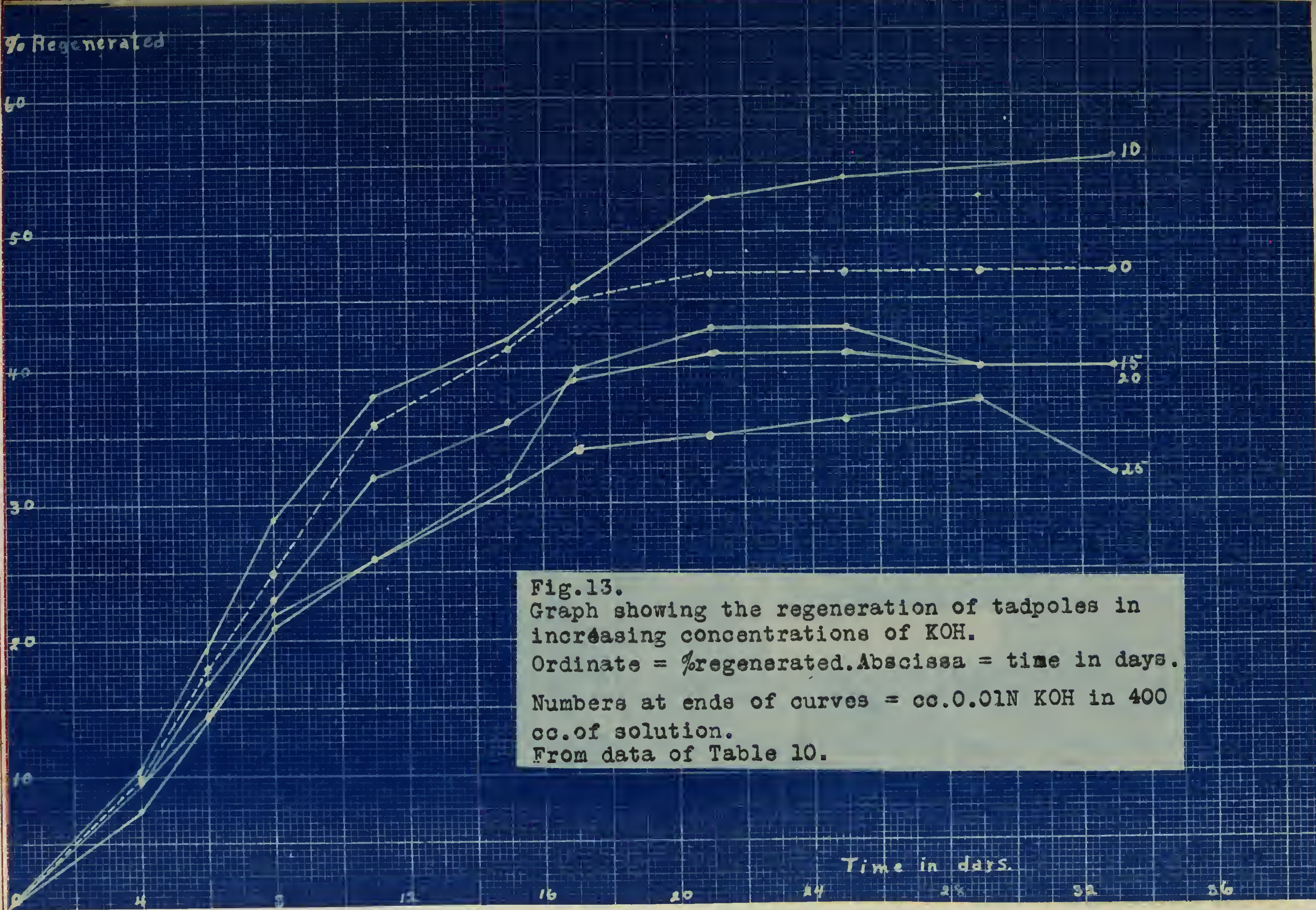


Fig.13.
Graph showing the regeneration of tadpoles in increasing concentrations of KOH.
Ordinate = %regenerated. Abscissa = time in days.

Numbers at ends of curves = cc.0.01N KOH in 400 cc.of solution.
From data of Table 10.

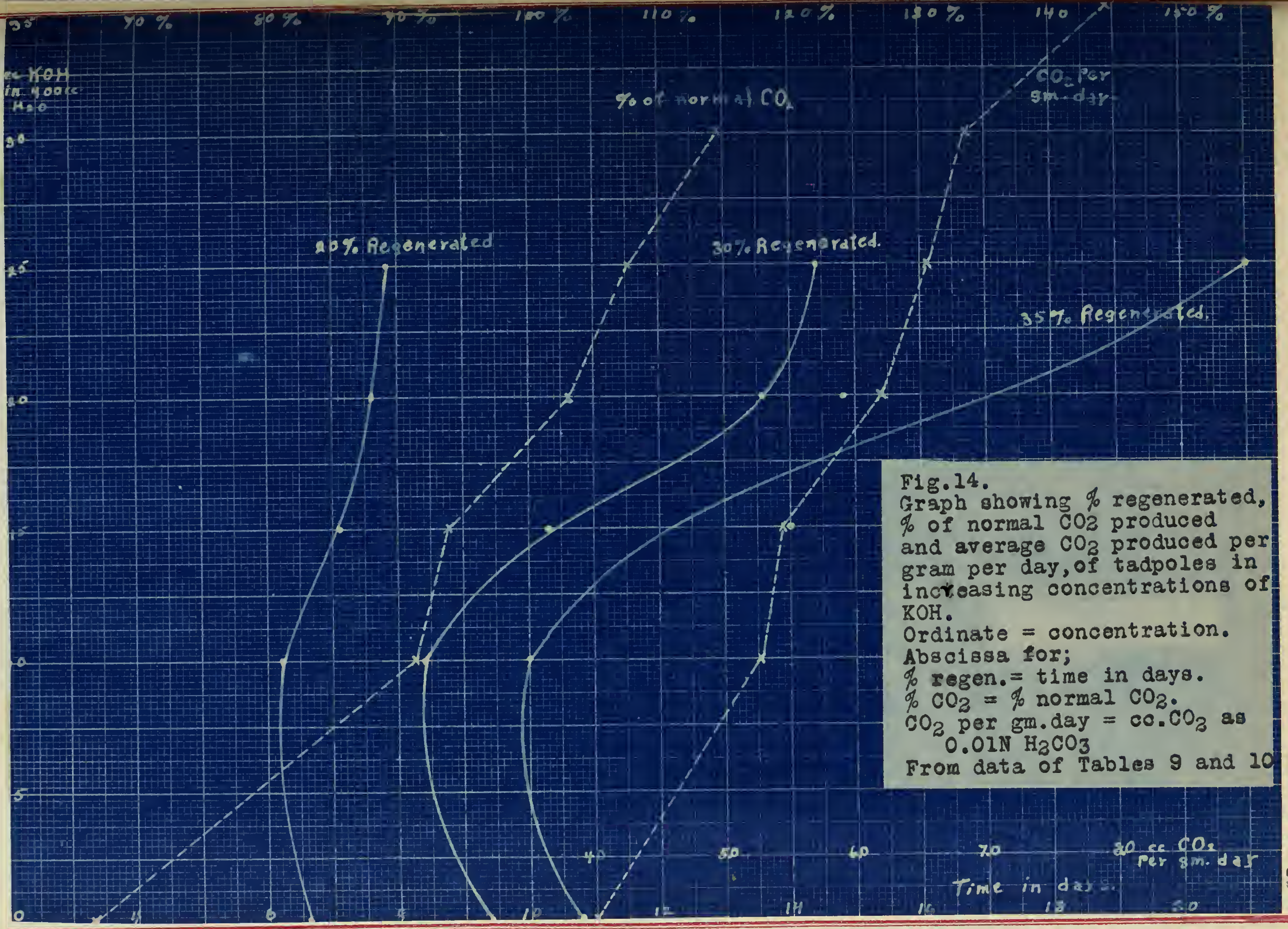


Fig.14.
Graph showing % regenerated, % of normal CO2 produced and average CO_2 produced per gram per day, of tadpoles in increasing concentrations of KOH.
Ordinate = concentration.
Abscissa for;
% regen. = time in days.
% CO_2 = % normal CO_2.
CO_2 per gm.day = cc.CO_2 as
0.01N H_2CO_3
From data of Tables 9 and 10

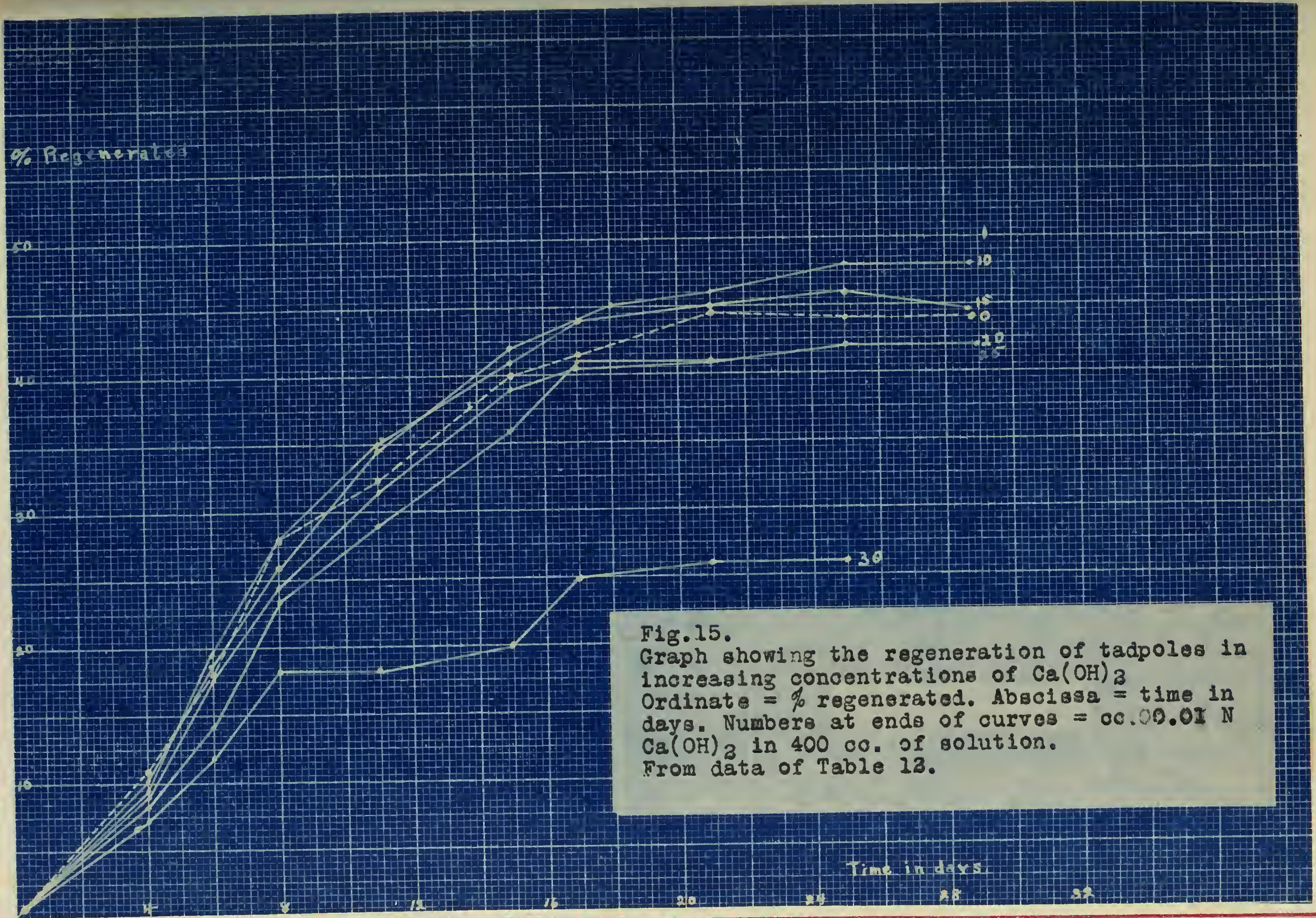


Fig.15.
Graph showing the regeneration of tadpoles in increasing concentrations of $Ca(OH)_2$
Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of curves = cc.00.01 N $Ca(OH)_2$ in 400 cc. of solution.
From data of Table 12.

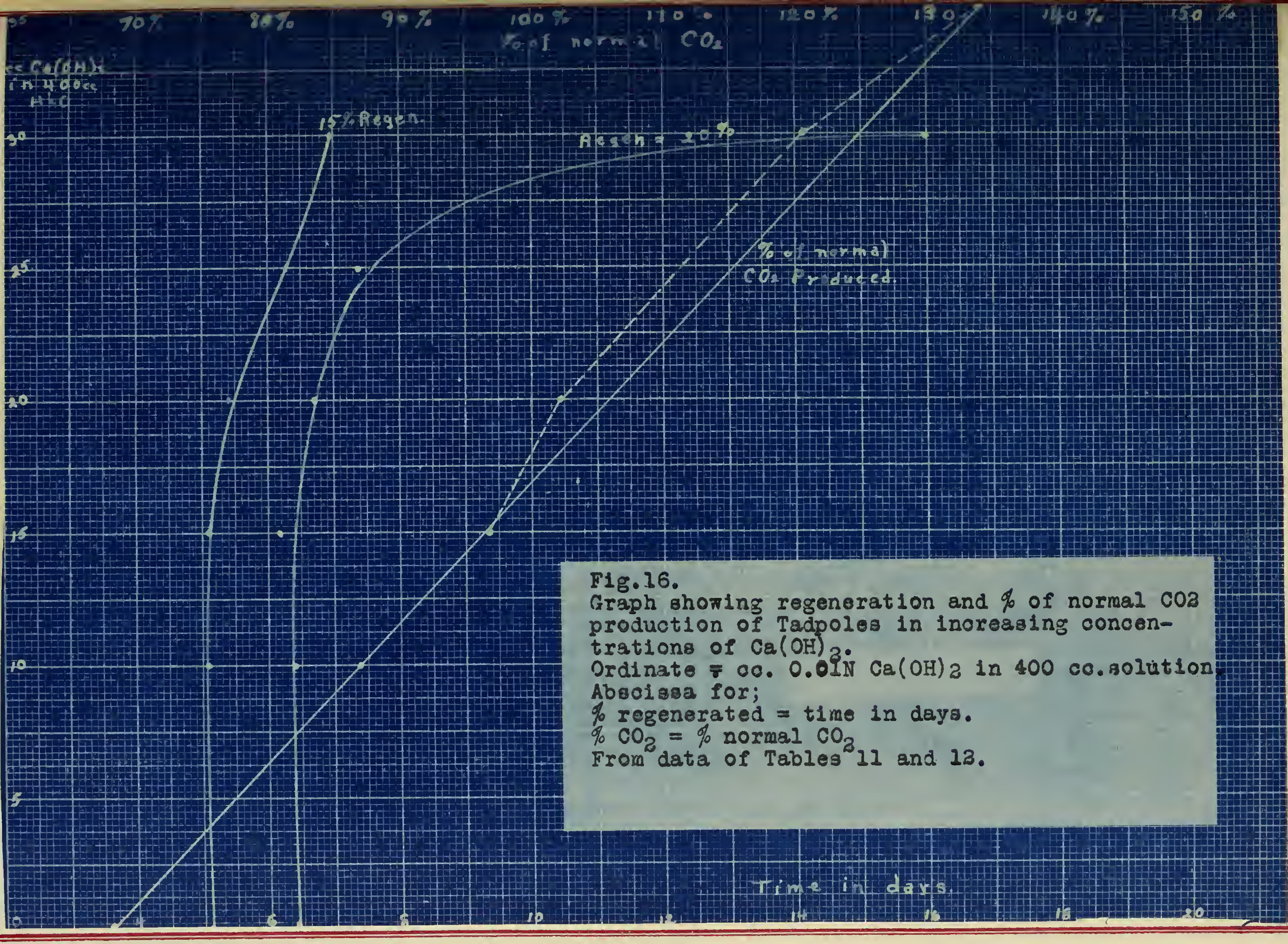


Fig.16.
Graph showing regeneration and % of normal CO2 production of Tadpoles in increasing concentrations of $Ca(OH)_2$.
Ordinate = cc. 0.01N Ca(OH)2 in 400 cc.solution.
Abscissa for;
% regenerated = time in days.
% CO_2 = % normal CO_2
From data of Tables 11 and 12.

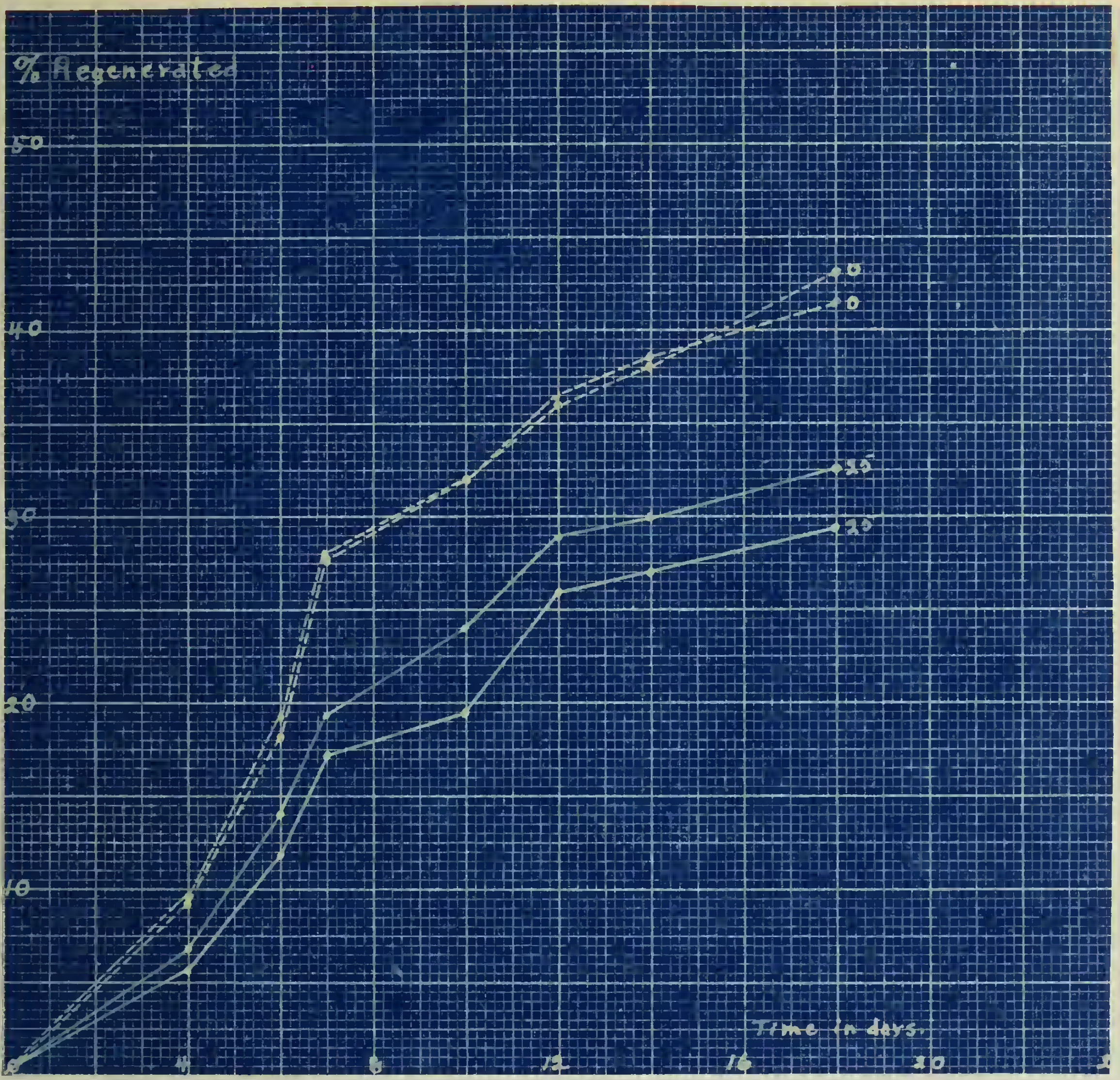


Fig.17.

Graph showing the regeneration of tadpoles in increasing concentrations of NaOH.
Ordinate = % regenerated.
Abscissa = time in days.
Numbers at ends of curves = cc. 0.01N NaOH
   per liter of solution.
From data of Table 13.

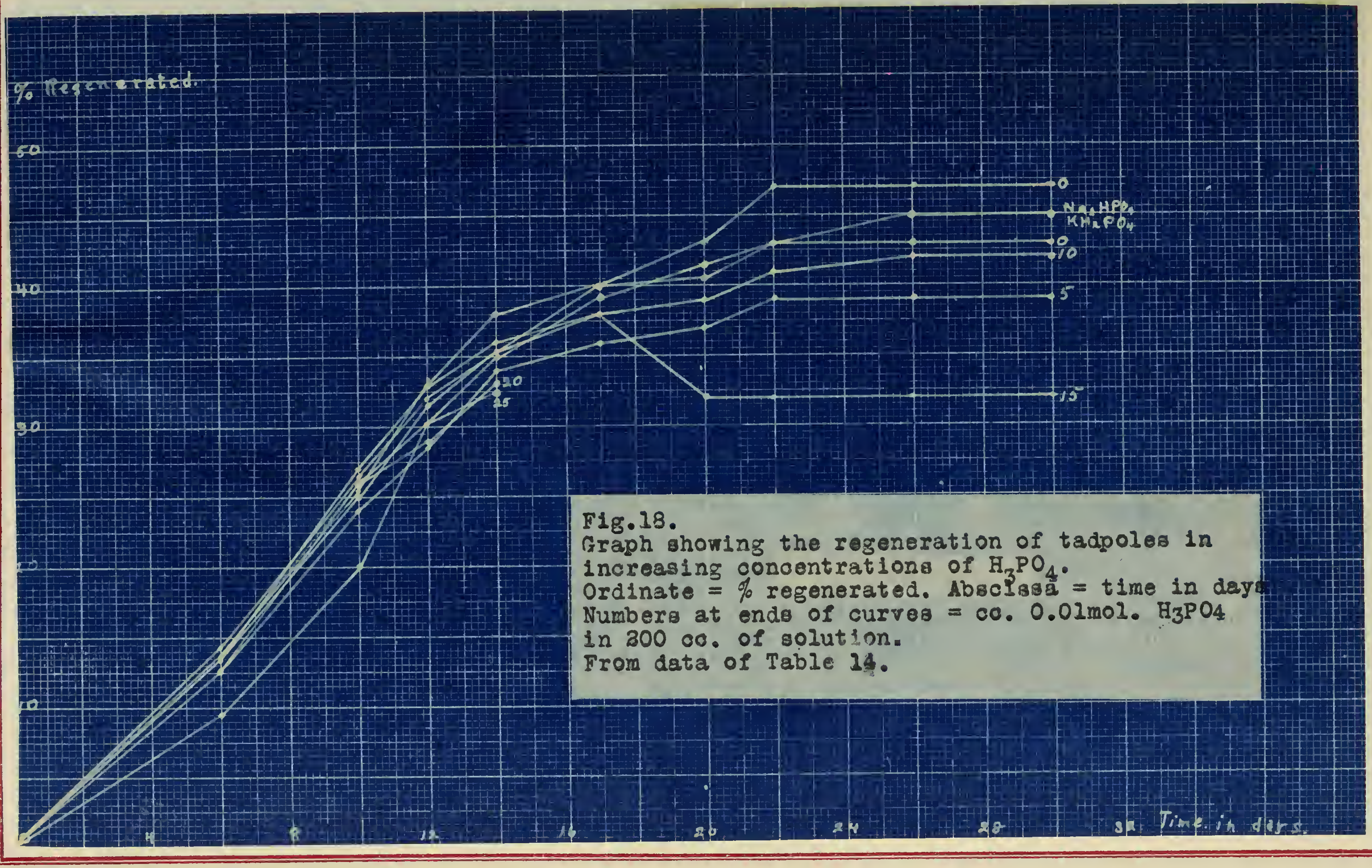


Fig.18.
Graph showing the regeneration of tadpoles in increasing concentrations of H_3PO_4.
Ordinate = % regenerated. Abscissa = time in days.
Numbers at ends of curves = cc. 0.01mol. H3PO4 in 200 cc. of solution.
From data of Table 14.

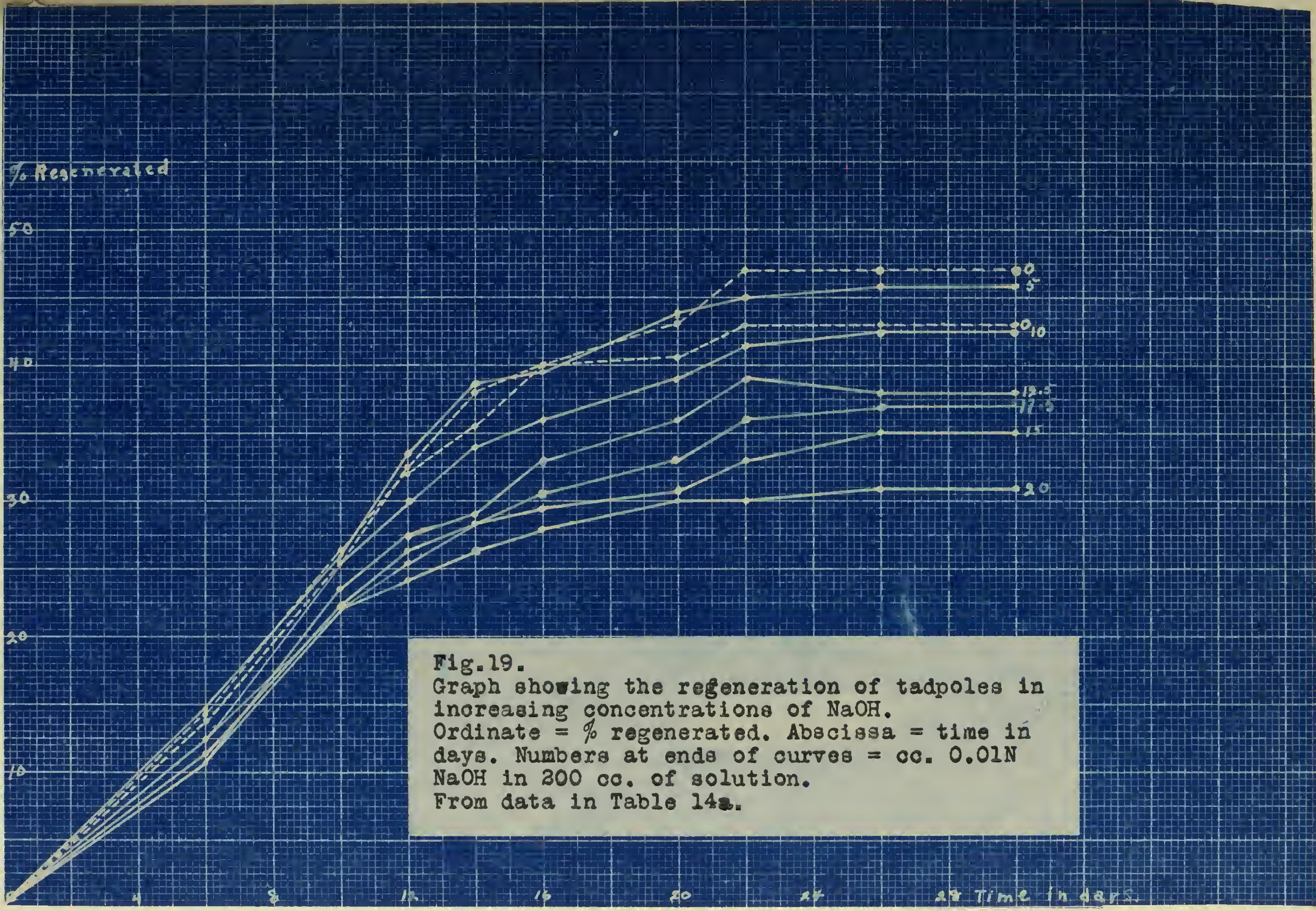


Fig. 19.
Graph showing the regeneration of tadpoles in increasing concentrations of NaOH.
Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of curves = cc. 0.01N NaOH in 200 cc. of solution.
From data in Table 14a.

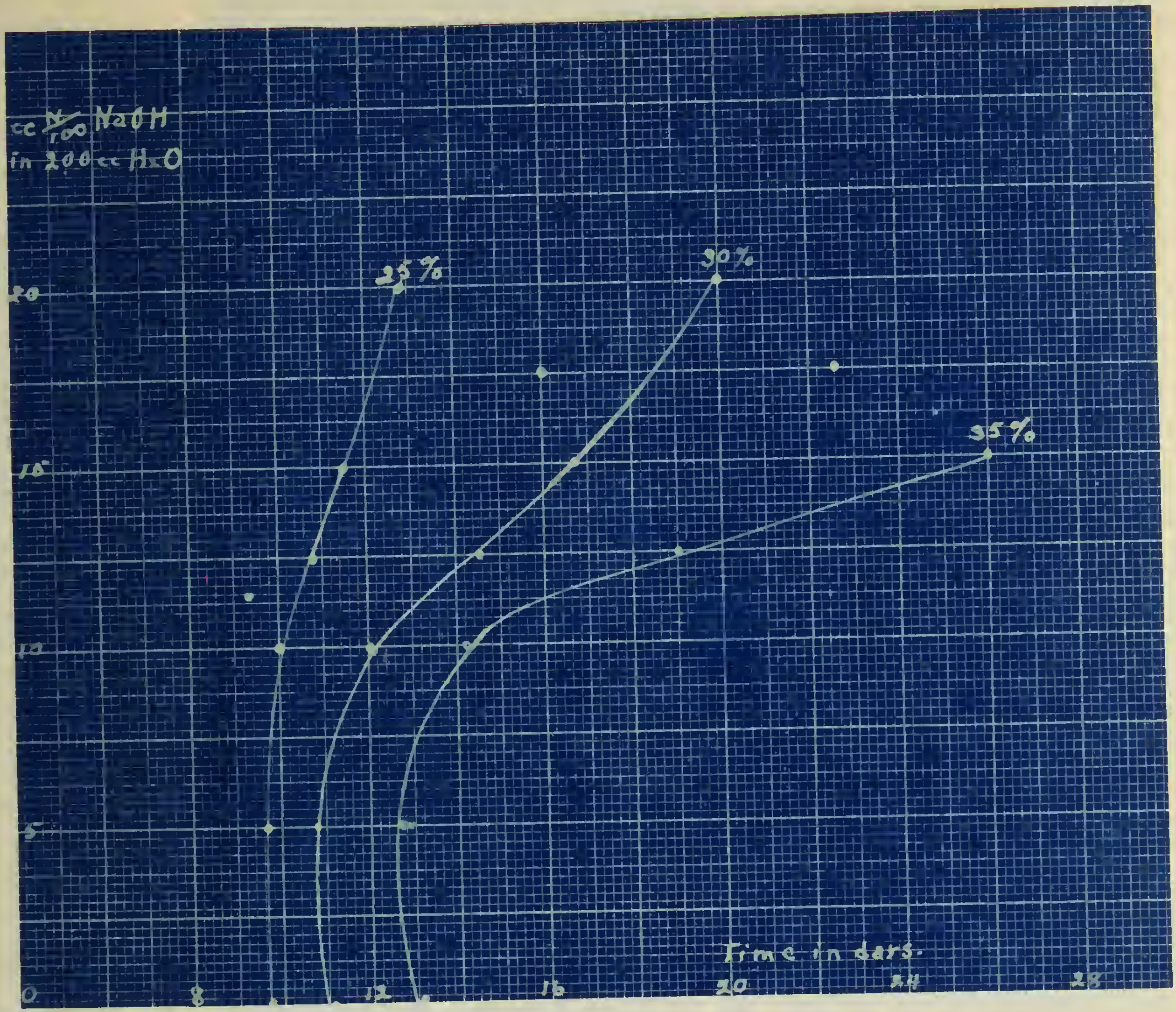


Fig.20.
Graph showing the regeneration of tadpoles in increasing concentrations of NaOH.
Ordinate = cc. 0.01N NaOH in 200 cc. solution.
Abscissa = time in days.
Numbers at ends of curves = % regenerated.
From data of Table 14a.

Fig.21.

Graph showing the regeneration of tadpoles in increasing concentrations of HNO_3. Ordinate = % reg3nerated. Abscissa = time in days. Numbers at ends of curves = cc. 0.01N HNO_3 in 200 cc. of solution. From data of Table 15.

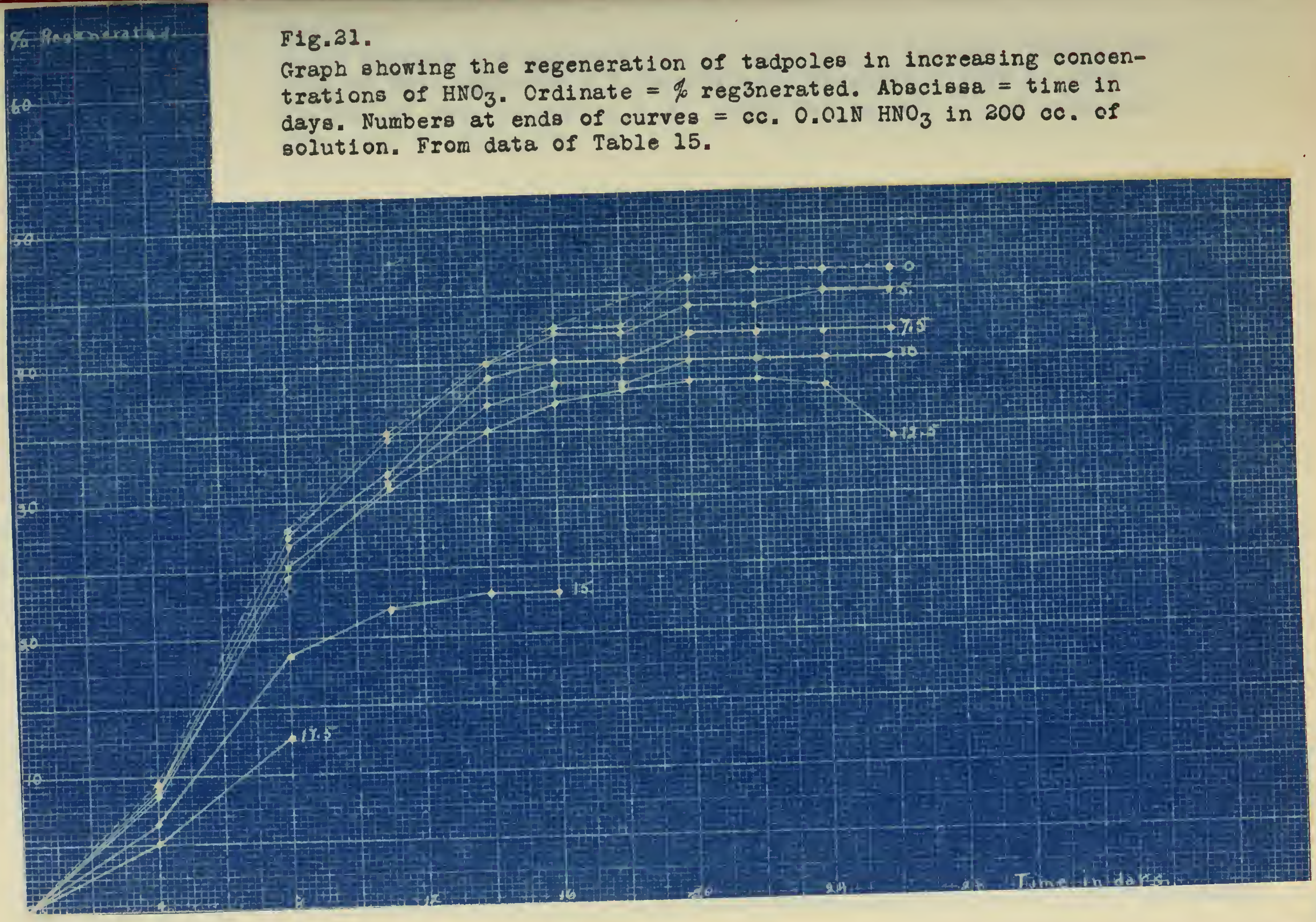
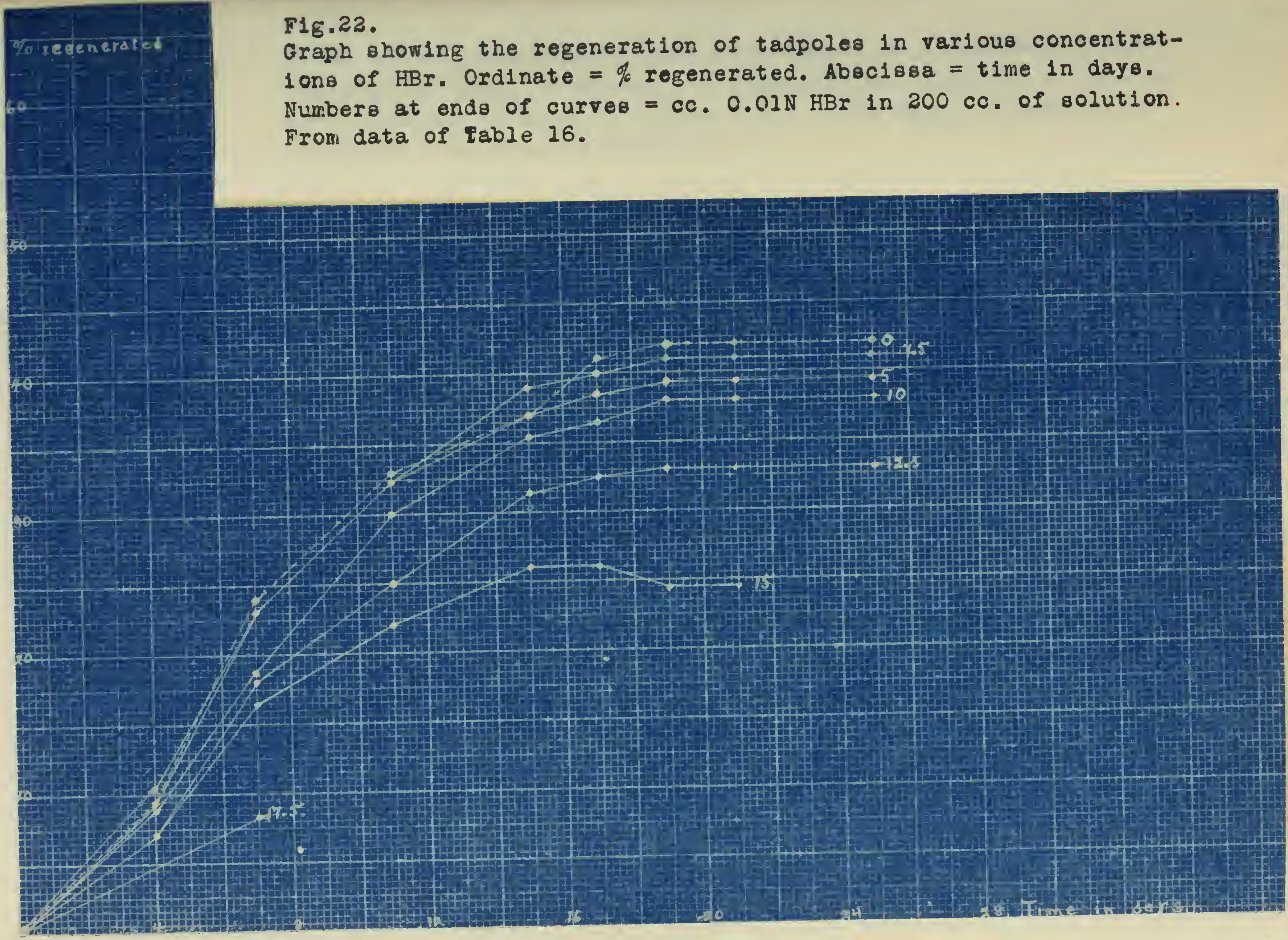


Fig.22.
Graph showing the regeneration of tadpoles in various concentrations of HBr. Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of curves = cc. 0.01N HBr in 200 cc. of solution. From data of Table 16.



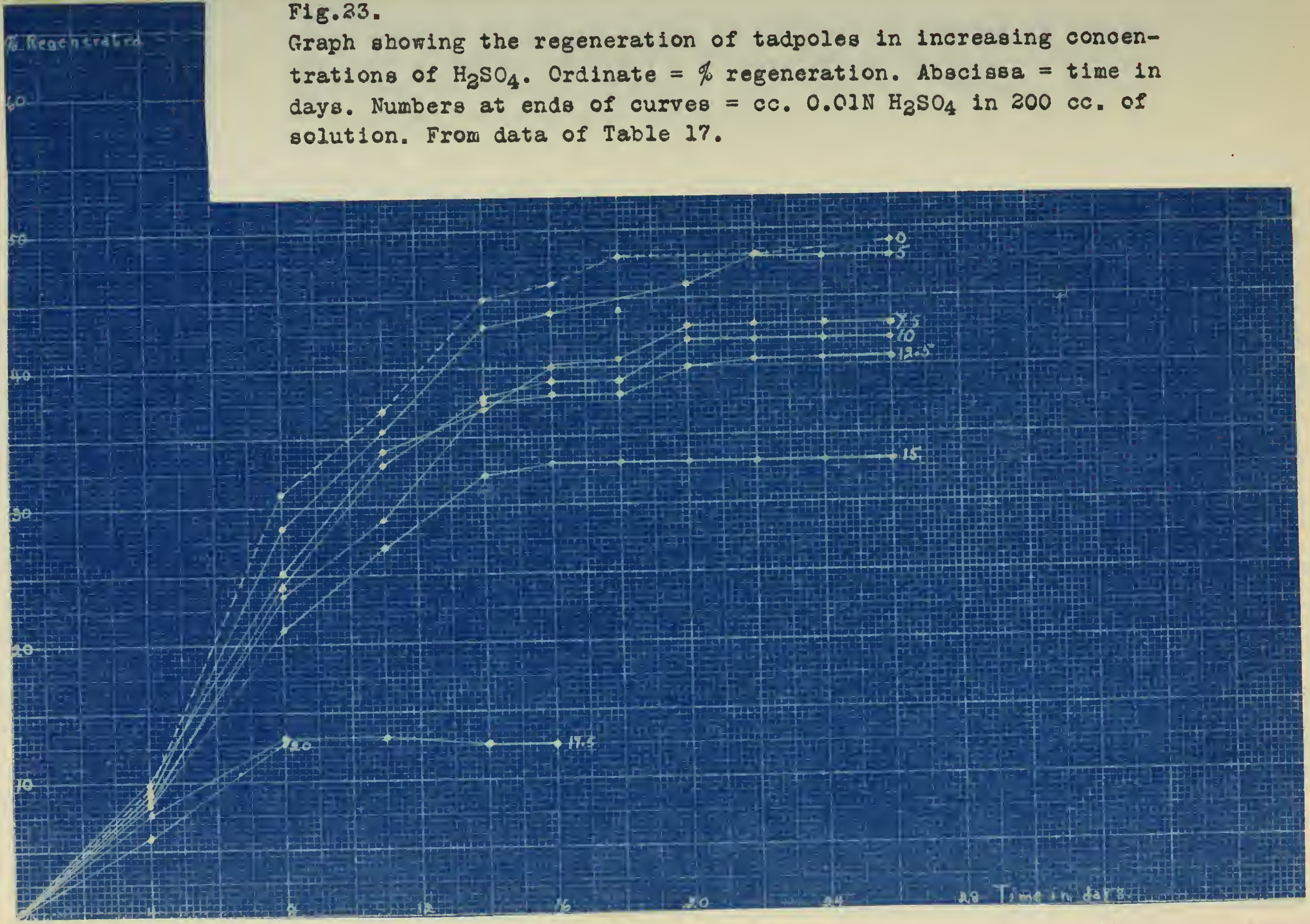


Fig.23.
Graph showing the regeneration of tadpoles in increasing concentrations of H_2SO_4. Ordinate = % regeneration. Abscissa = time in days. Numbers at ends of curves = cc. 0.01N H_2SO_4 in 200 cc. of solution. From data of Table 17.

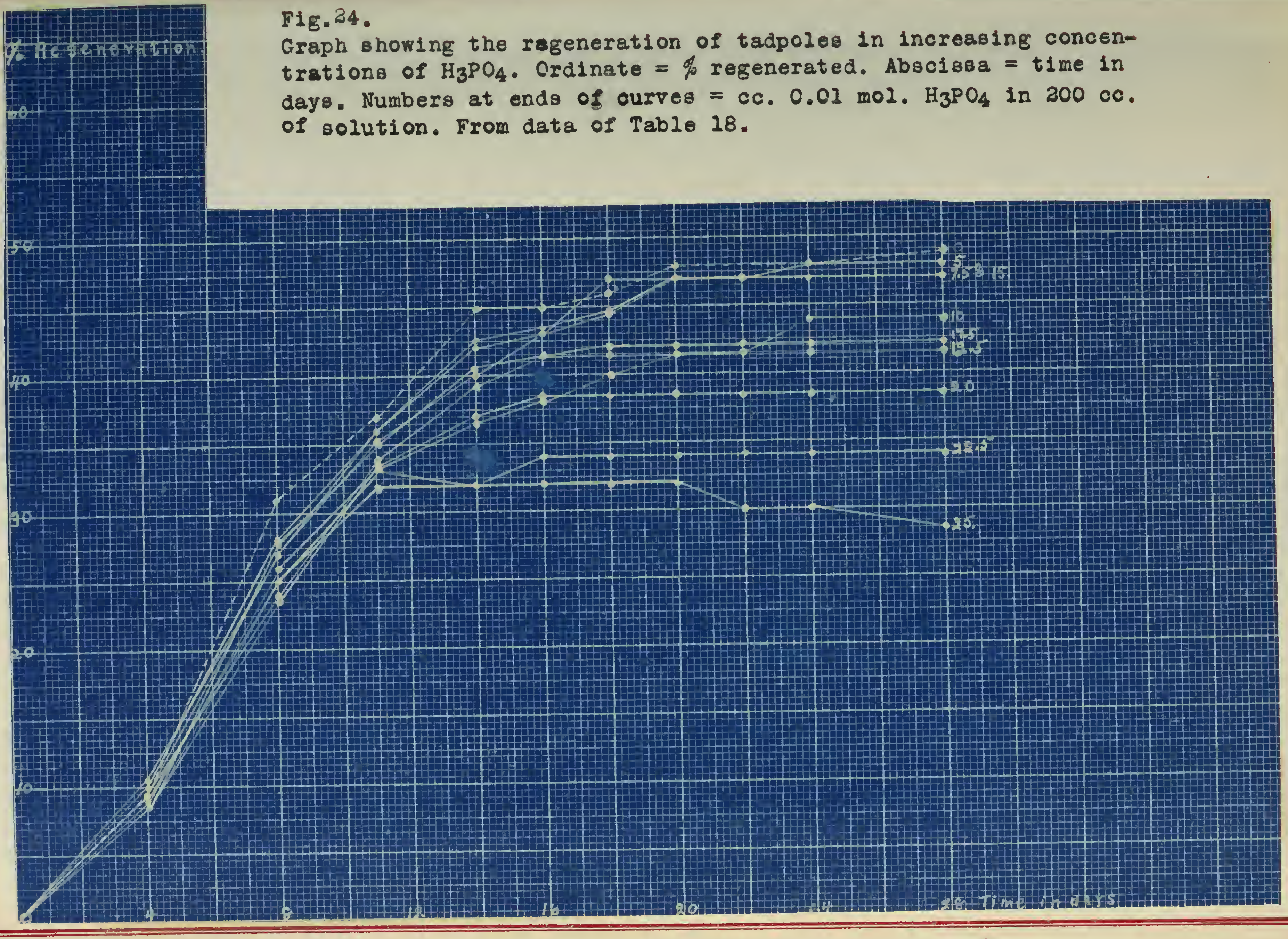


Fig. 24.
Graph showing the regeneration of tadpoles in increasing concentrations of H_3PO_4. Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of curves = cc. 0.01 mol. H_3PO_4 in 200 cc. of solution. From data of Table 18.

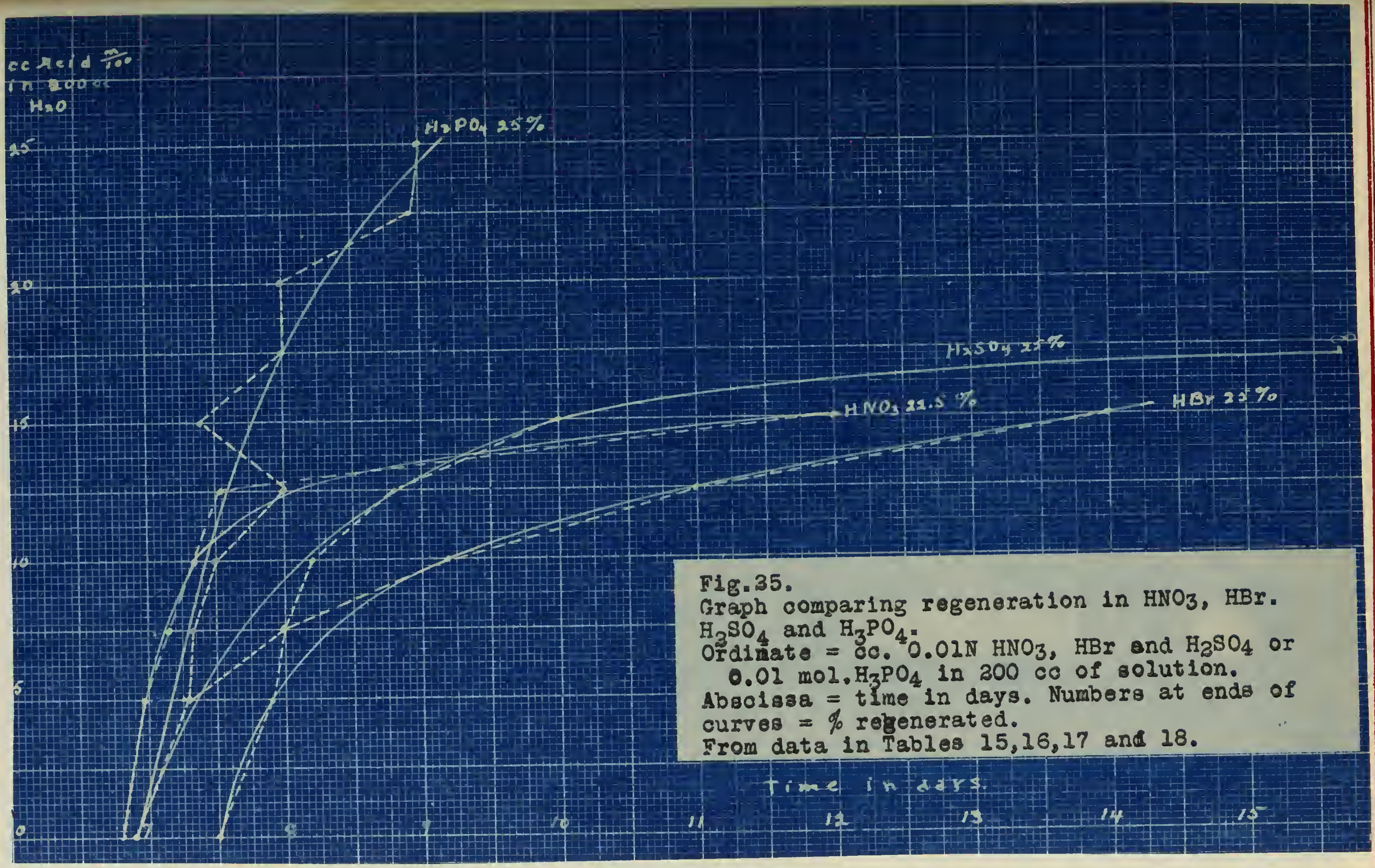


Fig.35.
Graph comparing regeneration in HNO_3, HBr.
H_2SO_4 and H_3PO_4.
Ordinate = cc. 0.01N HNO_3, HBr and H_2SO_4 or
0.01 mol.H_3PO_4 in 200 cc of solution.
Abscissa = time in days. Numbers at ends of
curves = % regenerated.
From data in Tables 15,16,17 and 18.

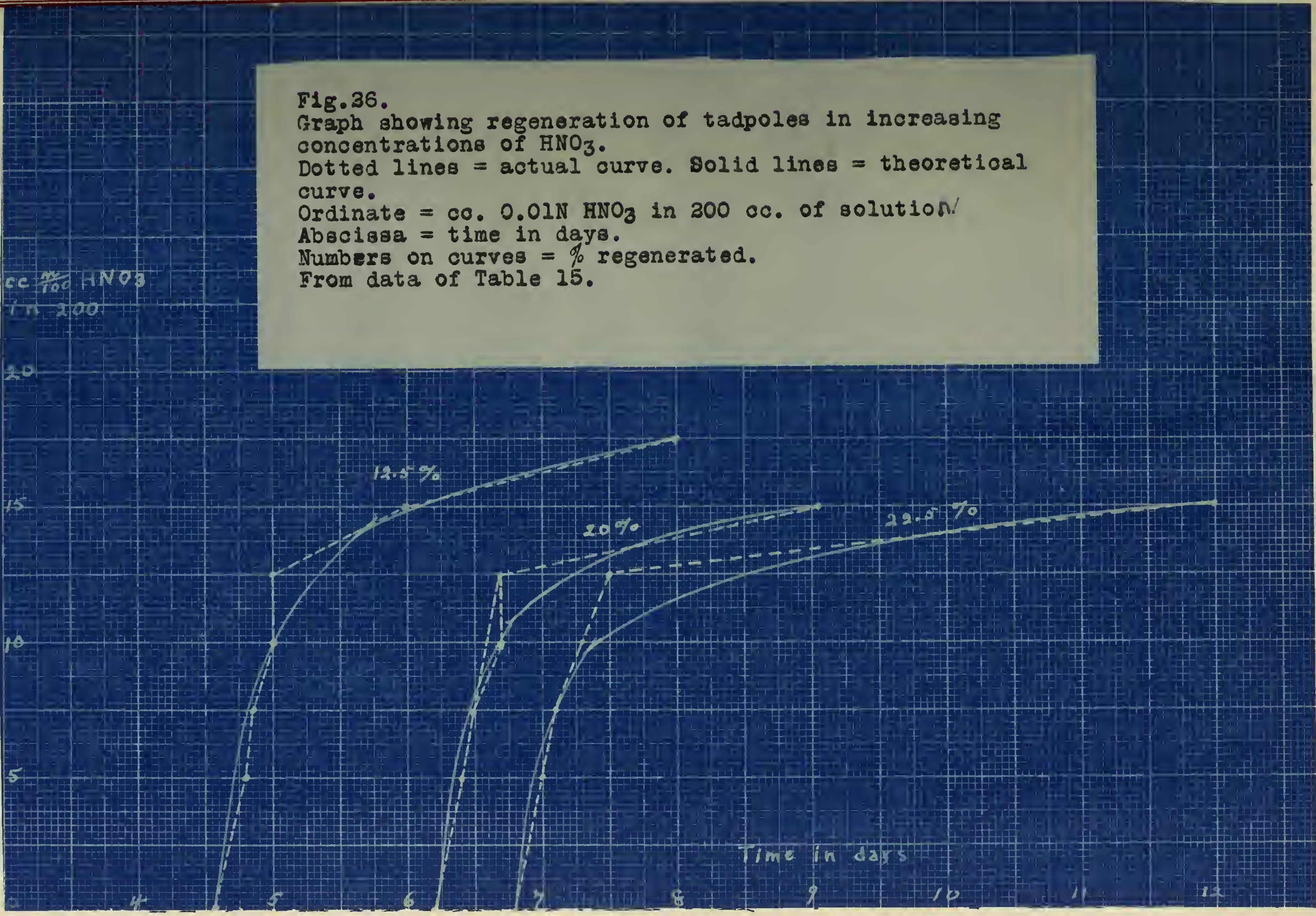


Fig.36.
Graph showing regeneration of tadpoles in increasing concentrations of HNO_3.
Dotted lines = actual curve. Solid lines = theoretical curve.
Ordinate = cc. 0.01N HNO_3 in 200 cc. of solution.
Abscissa = time in days.
Numbers on curves = % regenerated.
From data of Table 15.

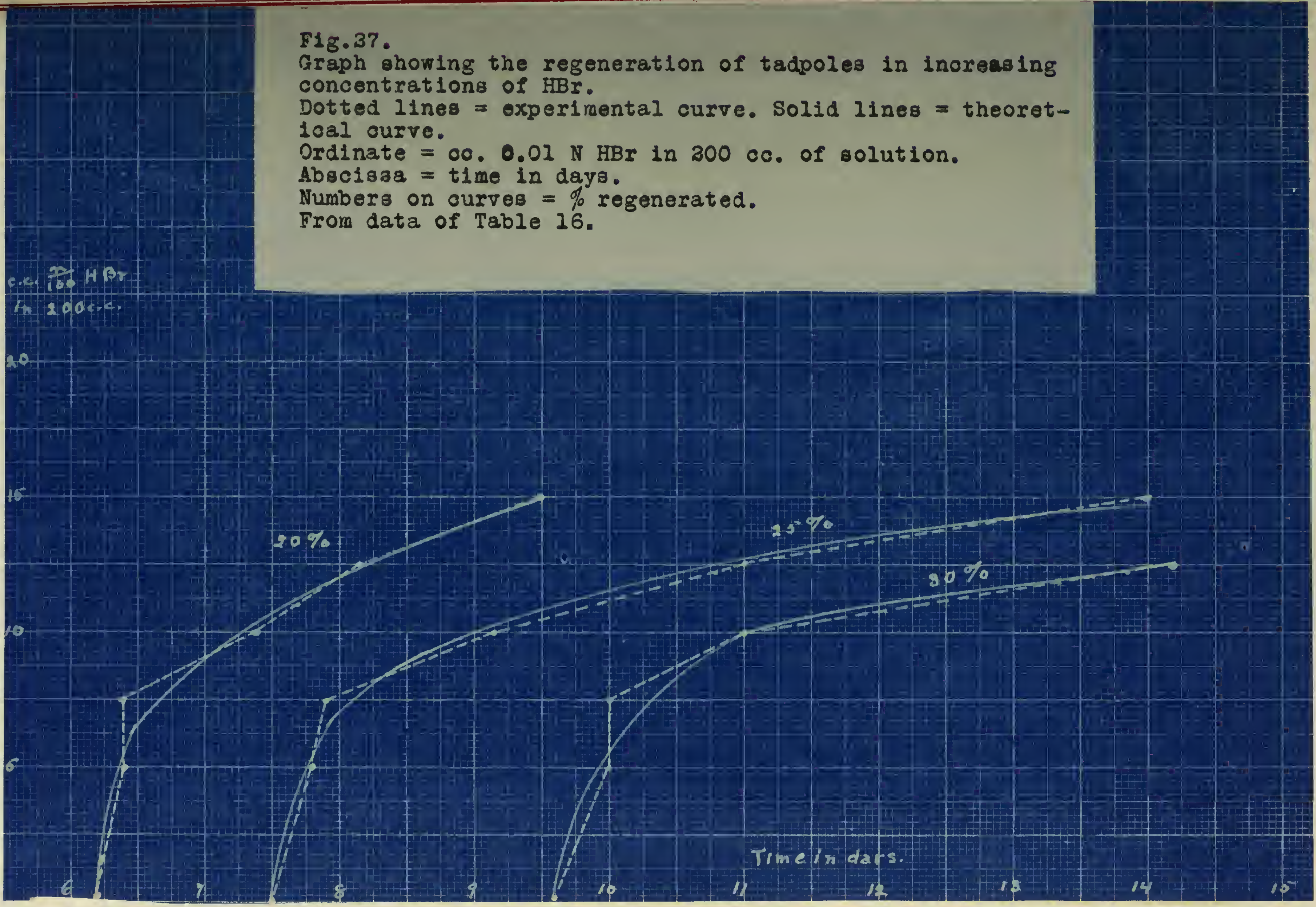


Fig.37.
Graph showing the regeneration of tadpoles in increasing concentrations of HBr.
Dotted lines = experimental curve. Solid lines = theoretical curve.
Ordinate = cc. 0.01 N HBr in 200 cc. of solution.
Abscissa = time in days.
Numbers on curves = % regenerated.
From data of Table 16.

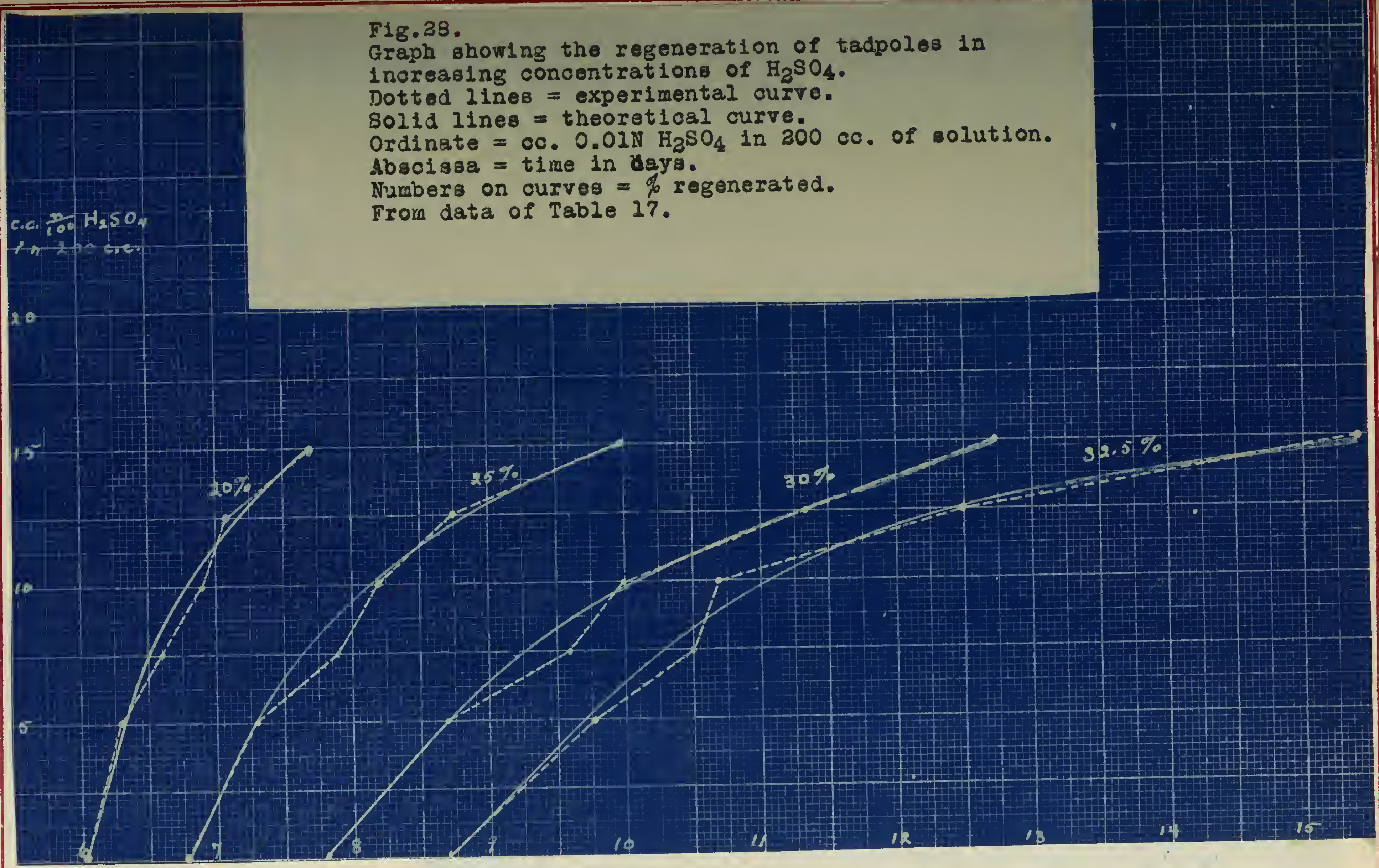


Fig.38.
Graph showing the regeneration of tadpoles in increasing concentrations of H_2SO_4.
Dotted lines = experimental curve.
Solid lines = theoretical curve.
Ordinate = cc. 0.01N H_2SO_4 in 200 cc. of solution.
Abscissa = time in days.
Numbers on curves = % regenerated.
From data of Table 17.

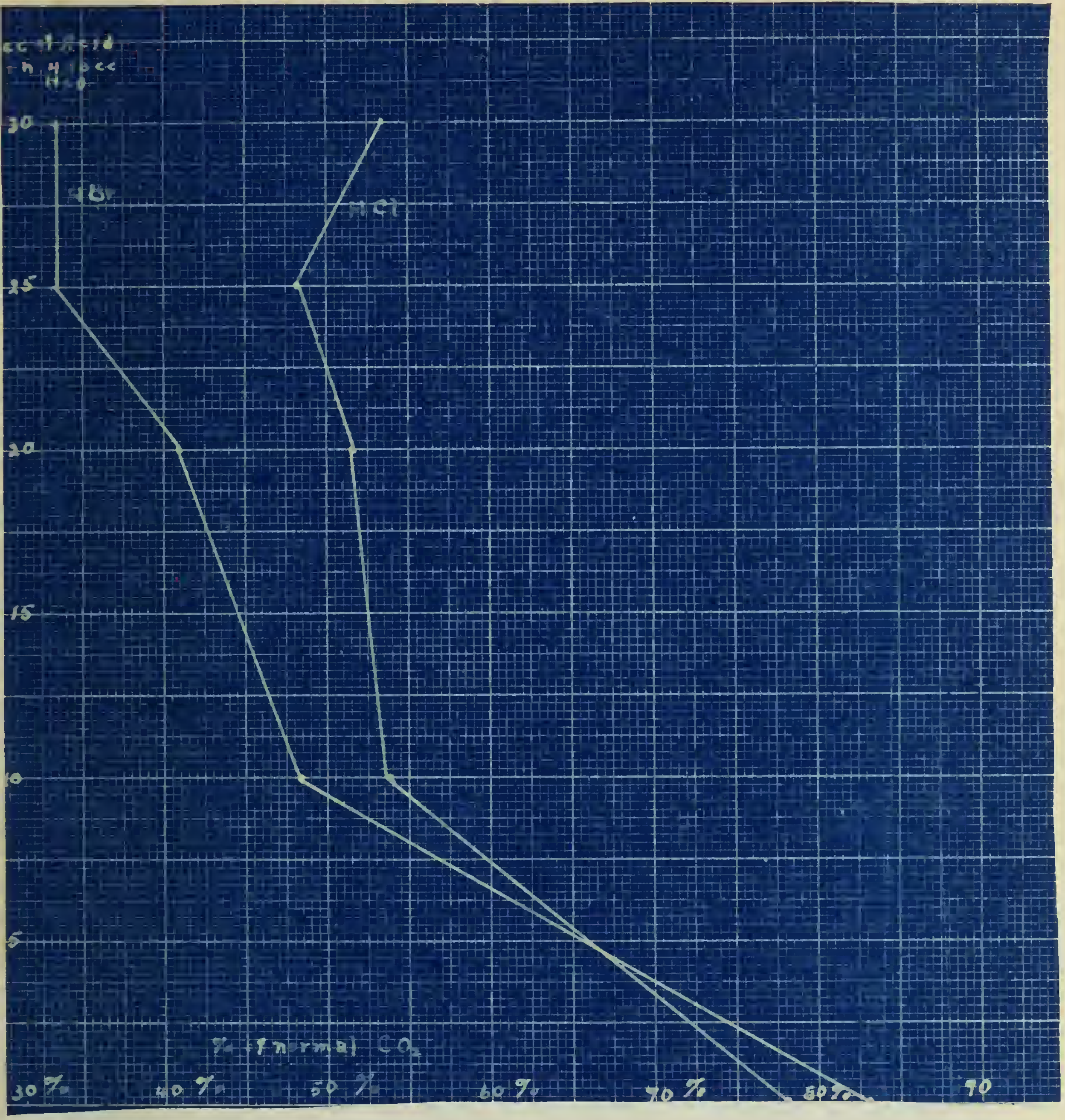


Fig.29.
Graph showing CO_2 production of tadpoles in increasing concentrations of HCl and HBr.
Ordinate = cc. 0.01N Acid in 400cc. of solution.
Abscissa = % normal CO_2 produced.
From data of Table 19.

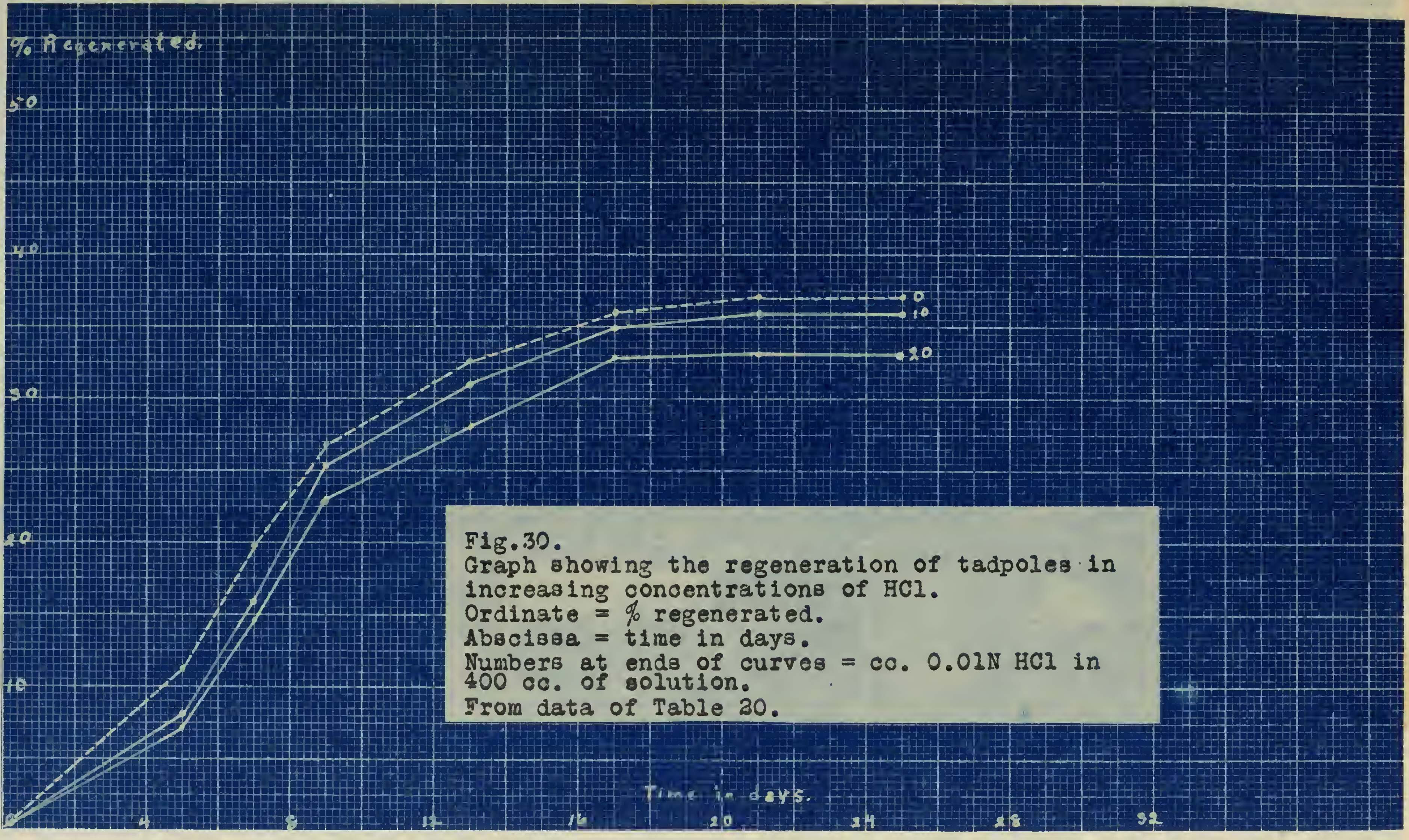
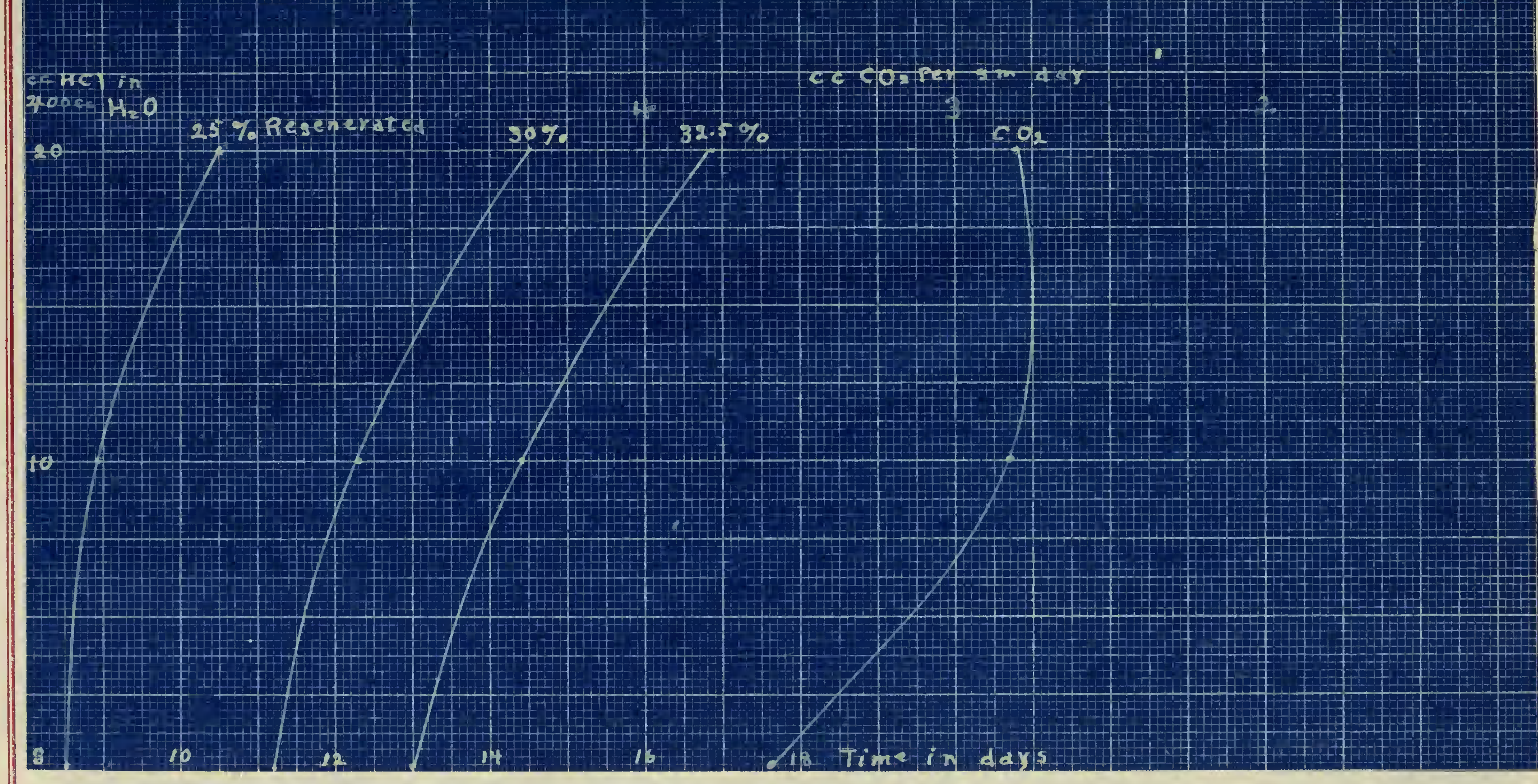


Fig.30.
Graph showing the regeneration of tadpoles in increasing concentrations of HCl.
Ordinate = % regenerated.
Abscissa = time in days.
Numbers at ends of curves = cc. 0.01N HCl in 400 cc. of solution.
From data of Table 20.

Fig.31.
Graph showing regeneration and CO_2 production in increasing concentrations of HCl.
Ordinate = cc. 0.01N HCl in 400 cc. of solution.
Abscissa; for % regenerated = time in days. For CO_2 produced = cc. CO_2 as 0.01N H_2CO_3
From data of Tables 19 and 20.



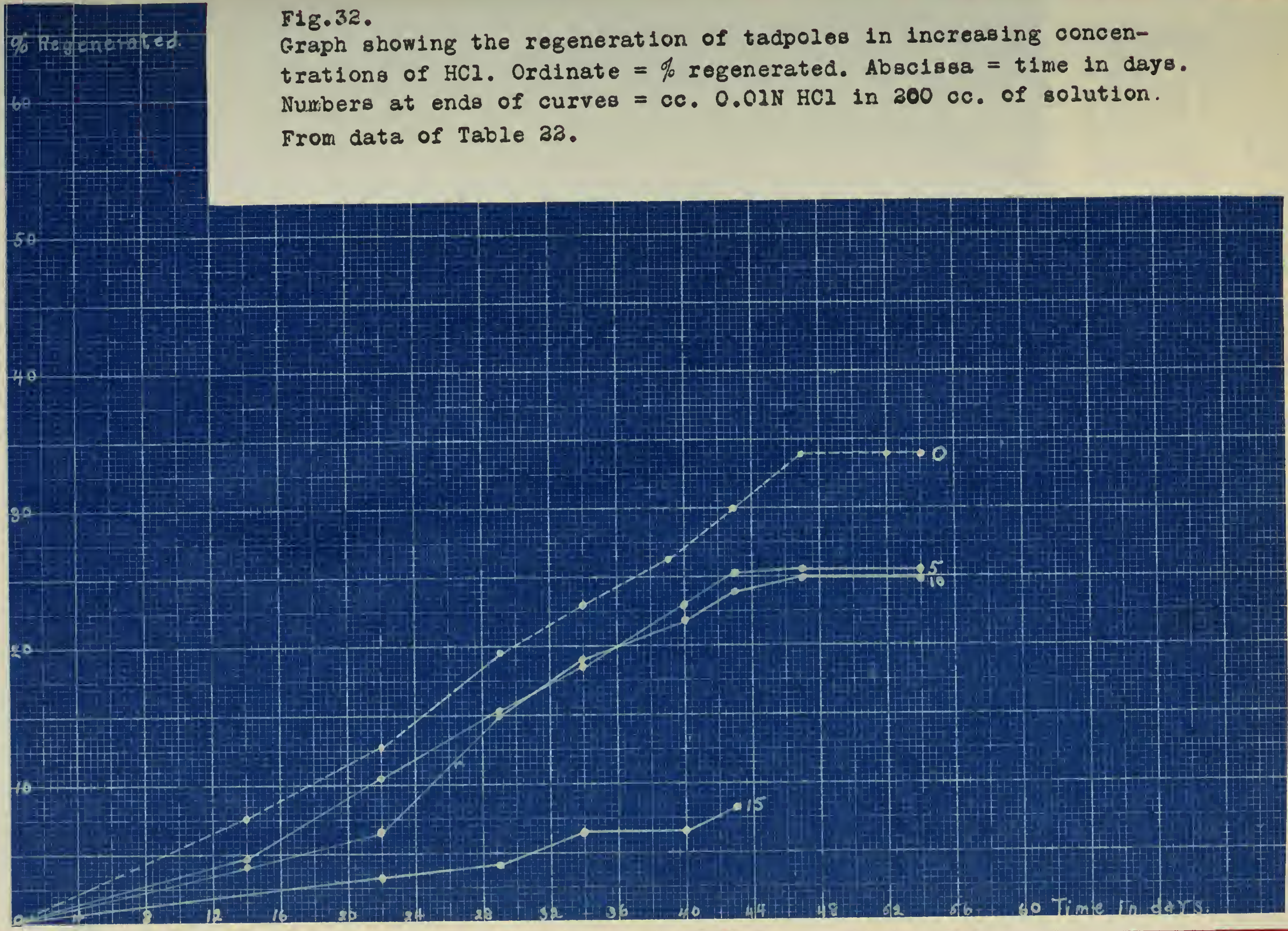
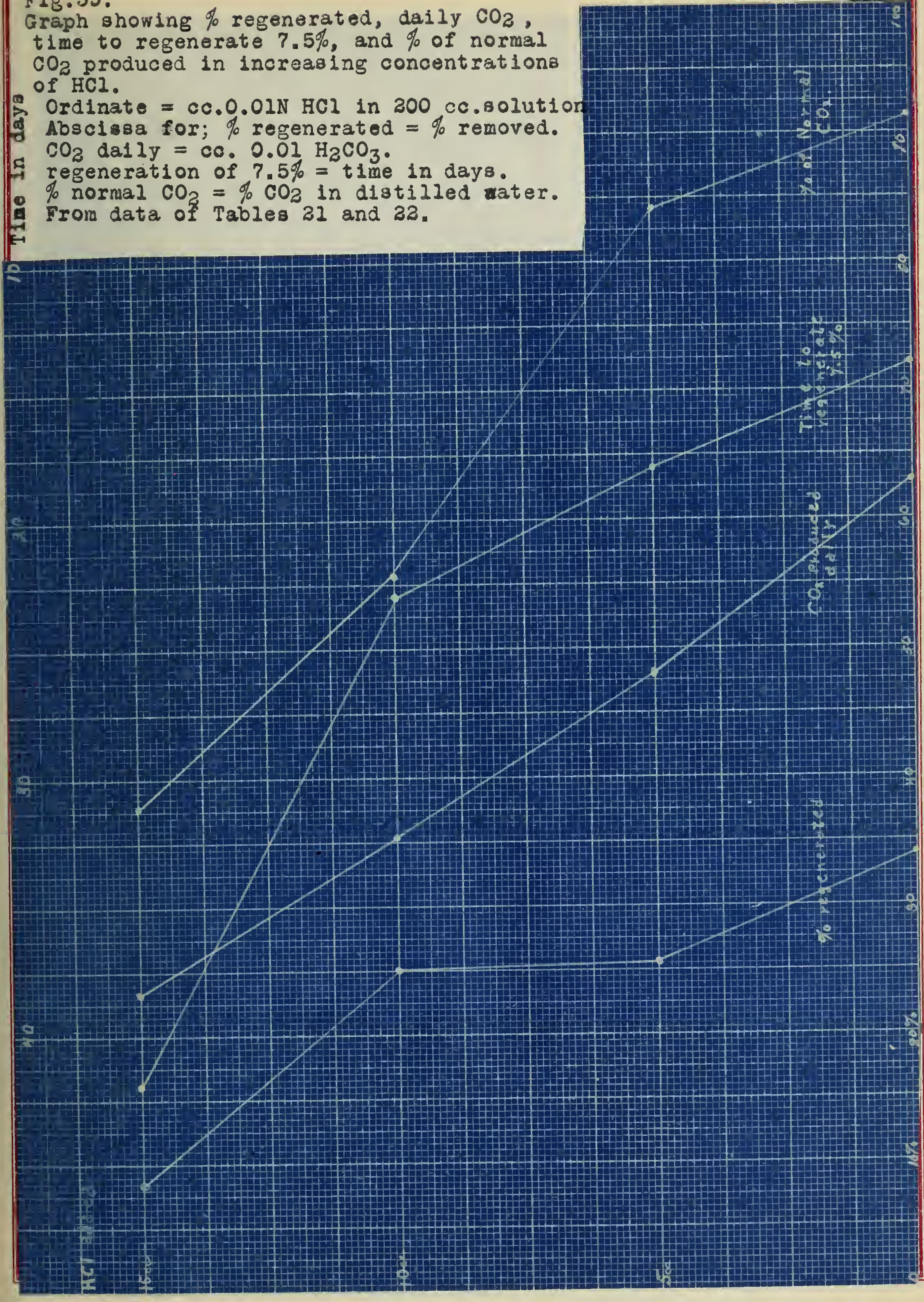


Fig.32.
Graph showing the regeneration of tadpoles in increasing concentrations of HCl. Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of curves = cc. 0.01N HCl in 200 cc. of solution.
From data of Table 22.

Fig.33.
Graph showing % regenerated, daily CO_2, time to regenerate 7.5%, and % of normal CO_2 produced in increasing concentrations of HCl.

Ordinate = cc.0.01N HCl in 200 cc.solution
Abscissa for; % regenerated = % removed.
CO_2 daily = cc. 0.01 H_2CO_3.
regeneration of 7.5% = time in days.
% normal CO_2 = % CO_2 in distilled water.
From data of Tables 21 and 22.



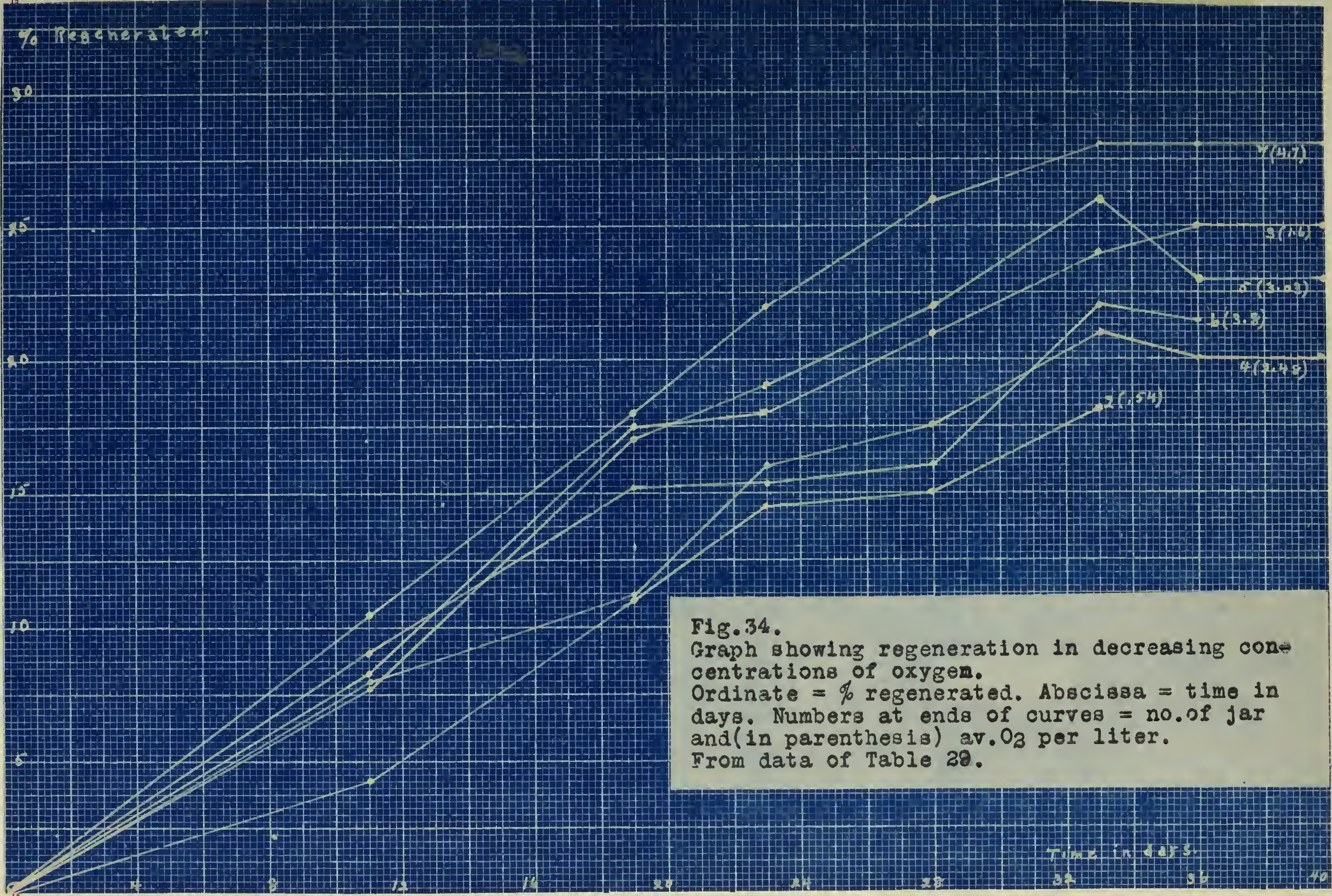


Fig.34.
Graph showing regeneration in decreasing concentrations of oxygem.
Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of curves = no.of jar and(in parenthesis) av.O_2 per liter.
From data of Table 29.

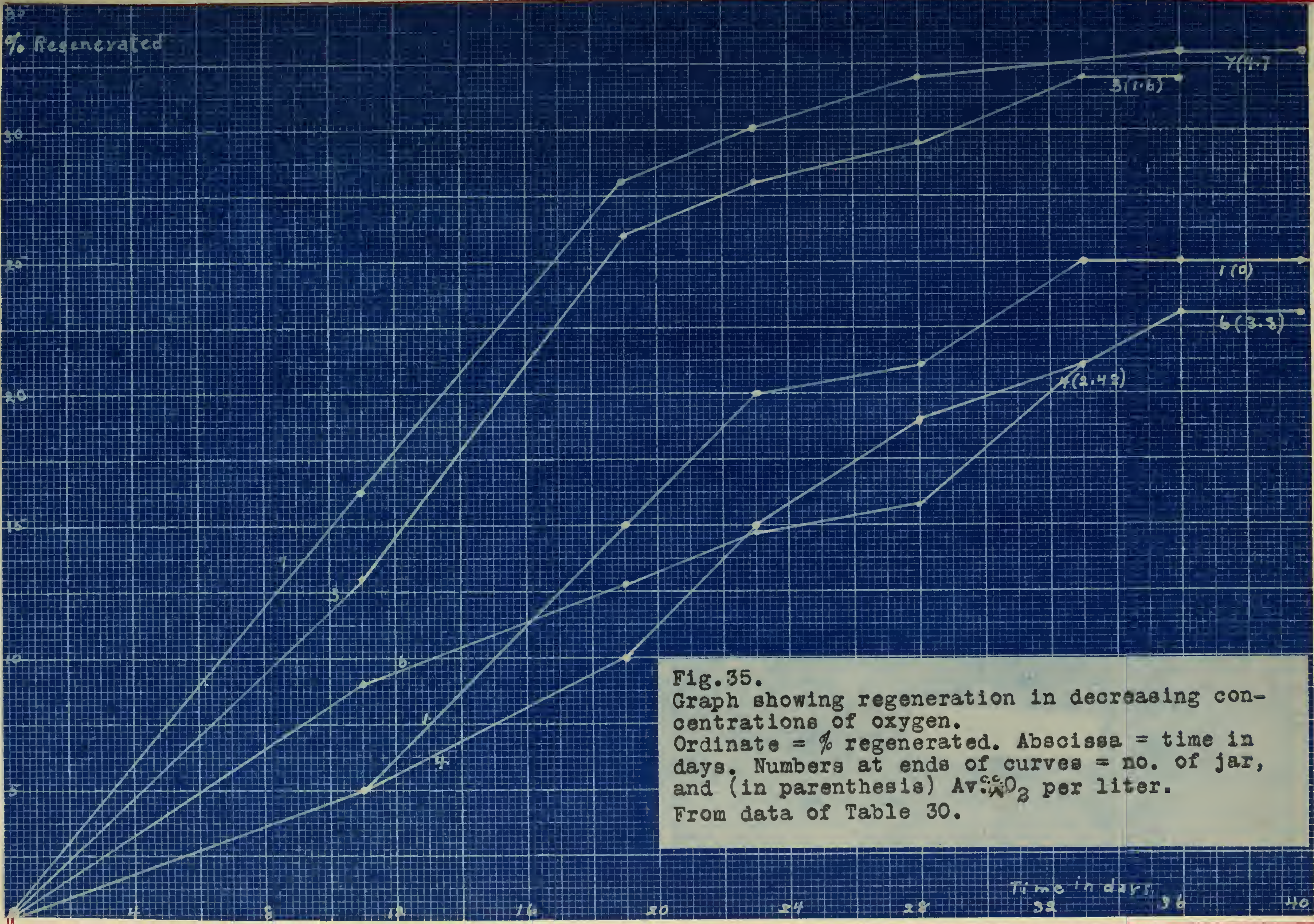


Fig.35.
Graph showing regeneration in decreasing concentrations of oxygen.
Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of curves = no. of jar, and (in parenthesis) Av. c.c. O_2 per liter.
From data of Table 30.

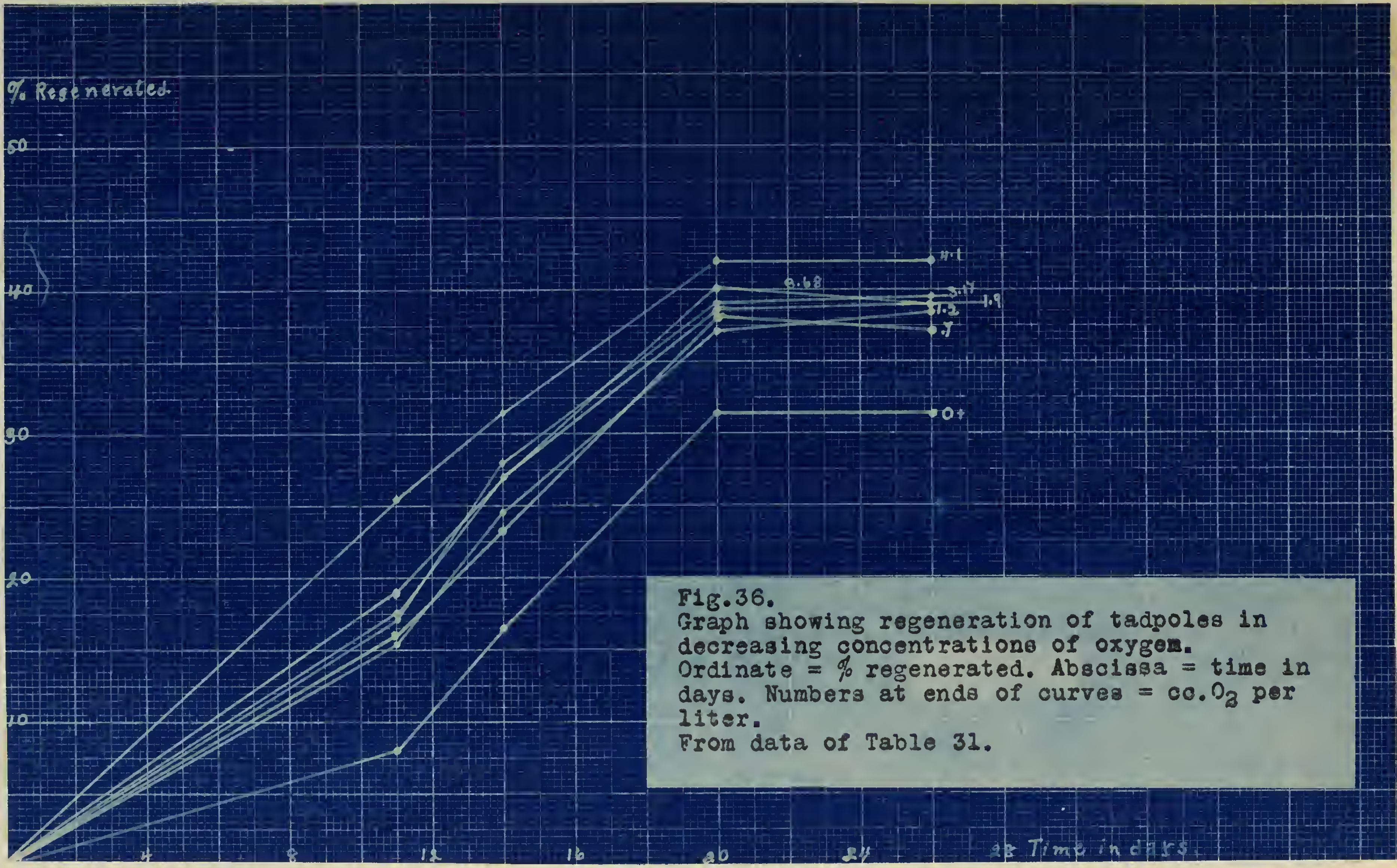


Fig.36.
Graph showing regeneration of tadpoles in decreasing concentrations of oxygem.
Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of curves = cc.O_2 per liter.
From data of Table 31.

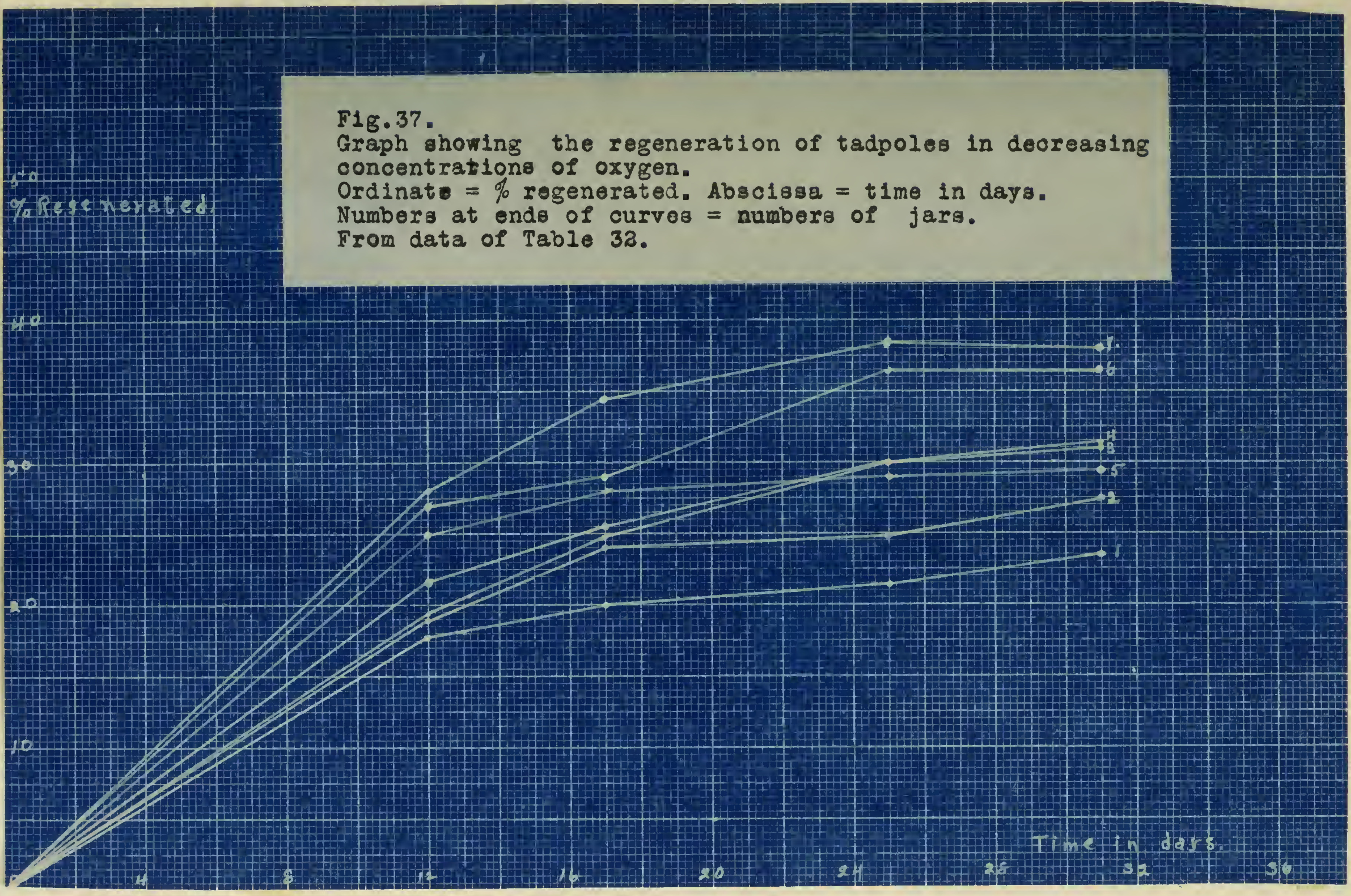


Fig.37.
Graph showing the regeneration of tadpoles in decreasing concentrations of oxygen.
Ordinate = % regenerated. Abscissa = time in days.
Numbers at ends of curves = numbers of jars.
From data of Table 32.

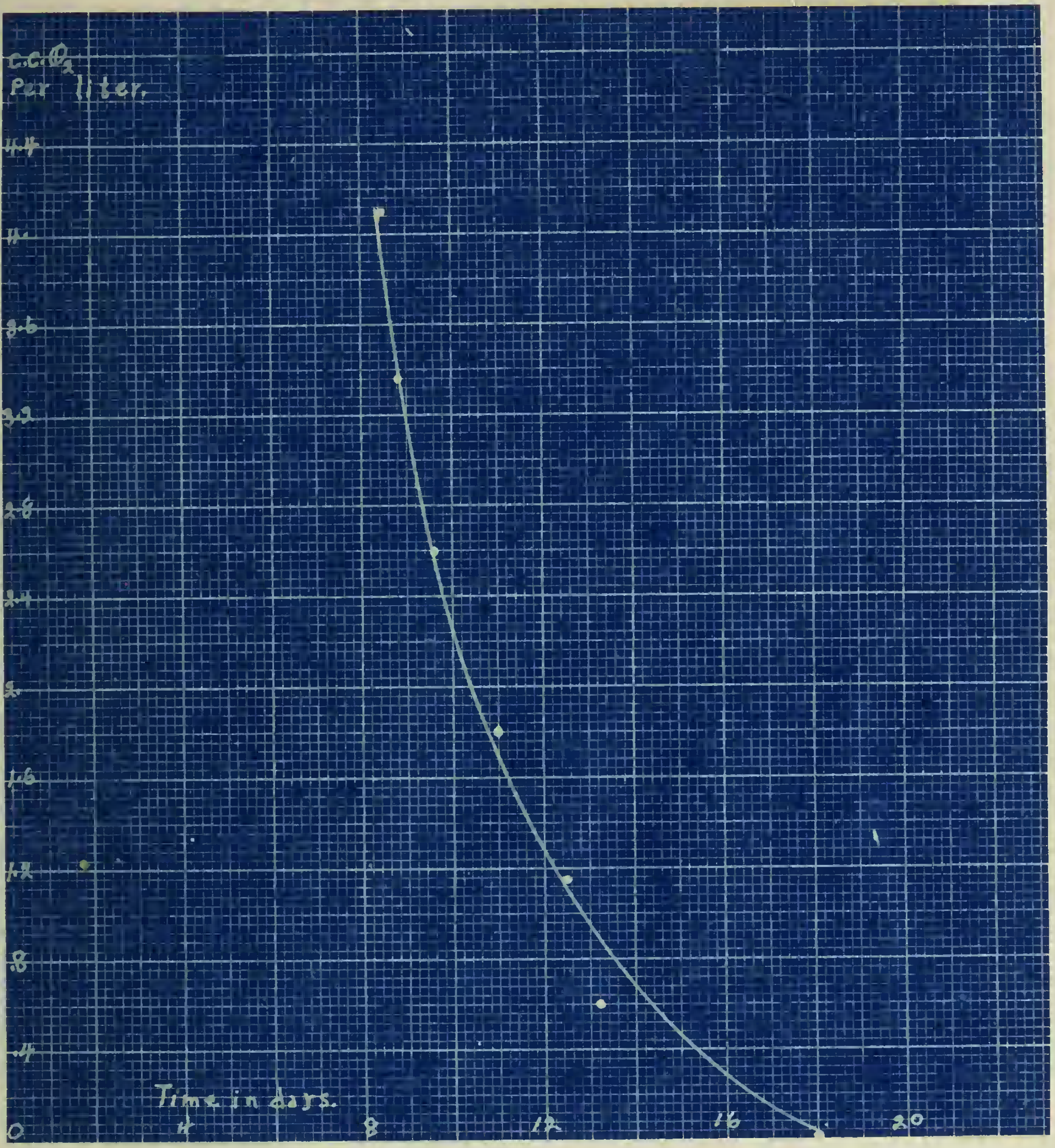


Fig. 38.
Graph showing the time required to regenerate 20% of the amount removed in decreasing concentrations of oxygen.
Ordinate = cc. oxygen per liter.
Abscissa = time in days.
From data of Table 32.

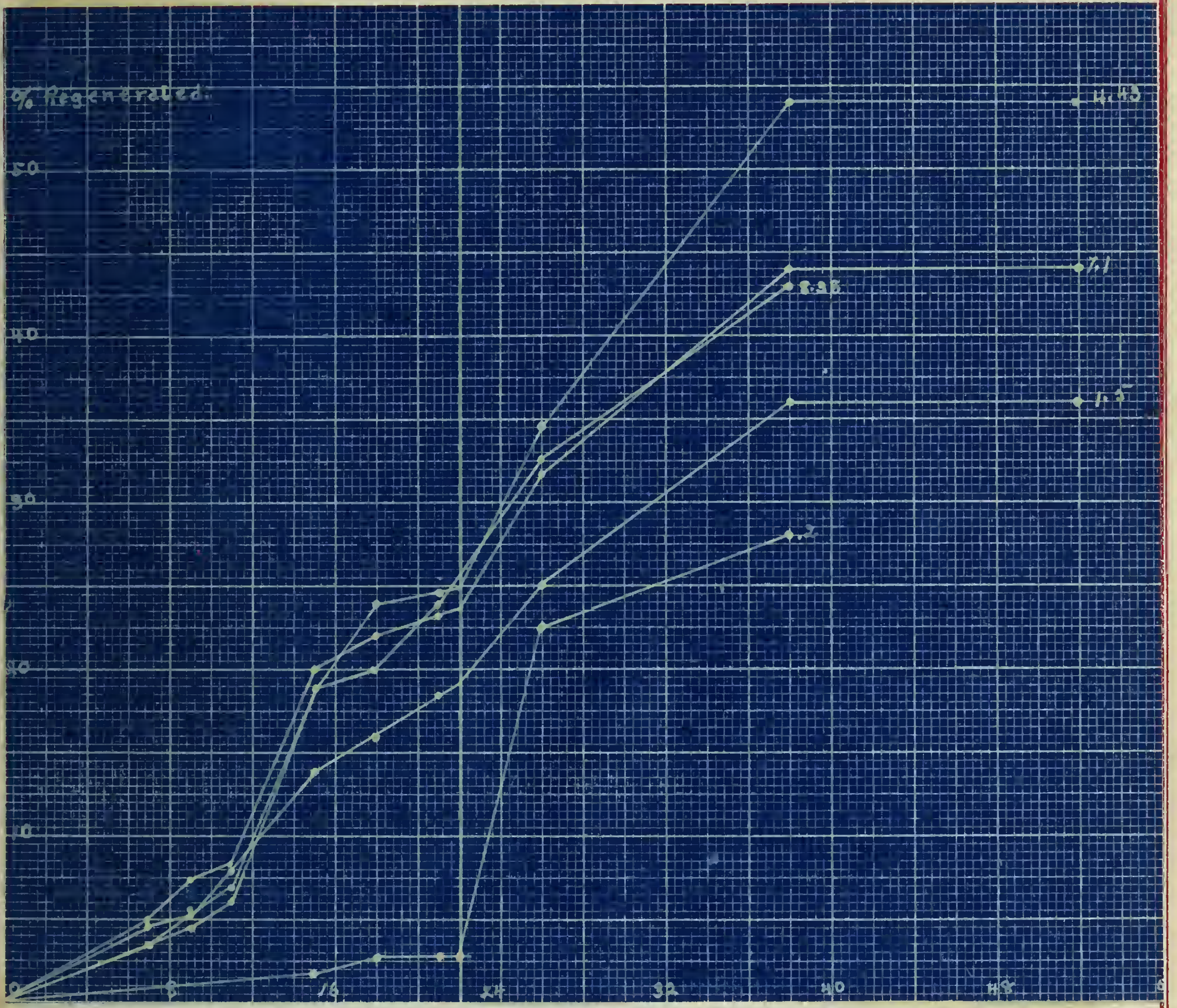


Fig.39.
Graph showing regeneration in decreasing concentrations of oxygen and after the water had been aerated. Heavy perpendicular line marks time of entrance of oxygen.
Ordinate = % regenerated. Abscissa = time in days. Numbers at ends of curves = cc.O_2 origionally in 1 liter of the water.
From data of Table 33.

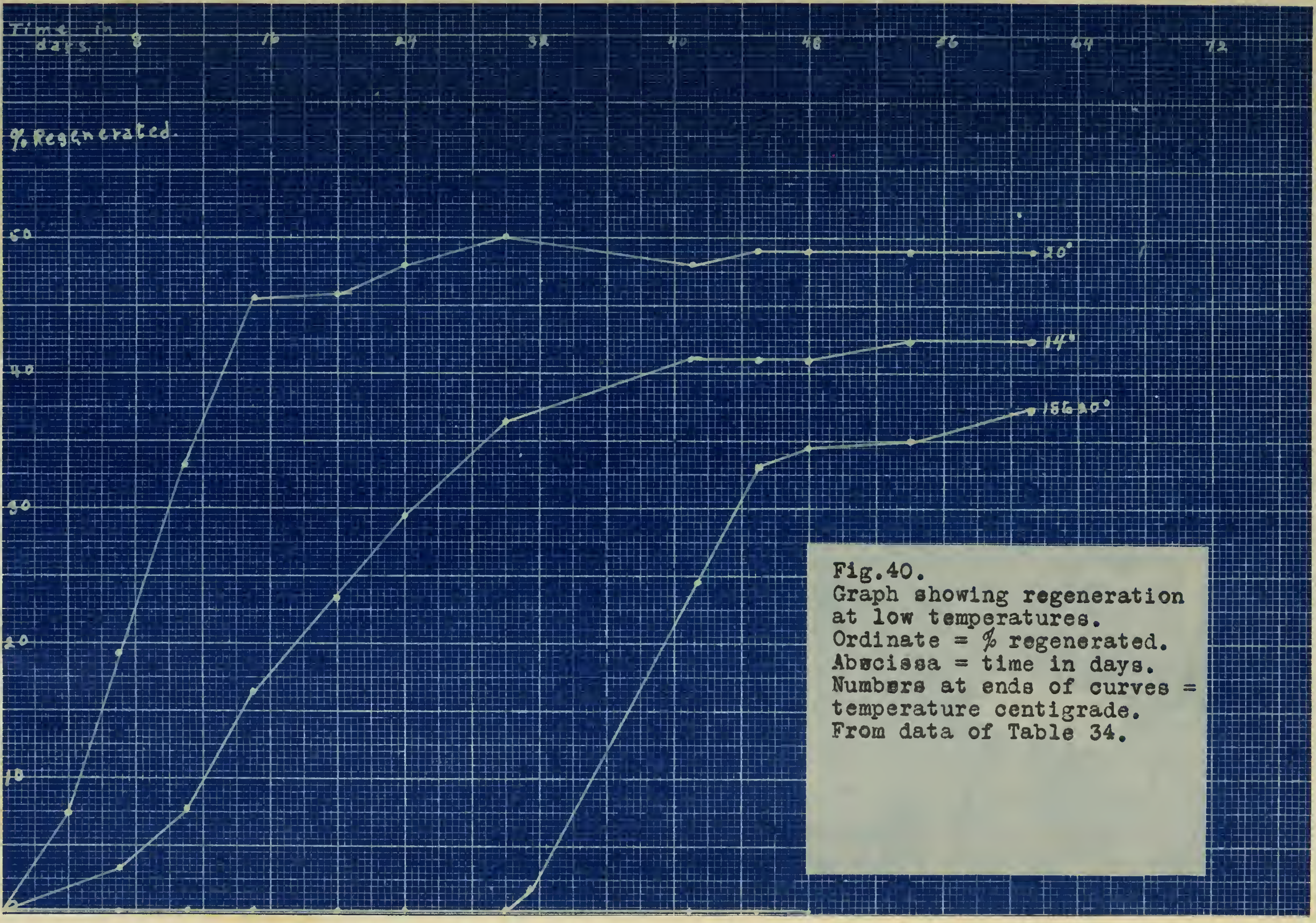


Fig.40.
Graph showing regeneration at low temperatures.
Ordinate = % regenerated.
Abscissa = time in days.
Numbers at ends of curves = temperature centigrade.
From data of Table 34.

VITA

Minna Ernestine Jewell was born near Irving, Kansas, February 9, 1892. She received her secondary education in the public high school of Irving, Kansas. In September 1910, she entered Colorado College, from which institution she received the degree A.B. in 1914. Her major was Biology. In the fall of 1914, she entered the Graduate School of the University of Illinois as a Scholar in Zoology, and the spring of 1915 she received the degree M.A. from the same institution. The years 1915-1916 and 1917-1918 were spent as a Graduate Fellow in the Department of Zoology of the University of Illinois, and the year 1916-1917 as a Graduate Assistant. The summer of 1916 was spent at the Pugent Sound Biological Station. Miss Jewell is a member of Sigma Xi and Iota Sigma Pi.

Publications

1916. Cylindrotaenia Americana Nov. Spec. From the Cricket Frog. Jour. Parasitol., 2:181-192.

1918. The Occurrence of Sichenase in the Digestive Tract of Invertebrates. Jour. Biol. Chem., 33:161-167. By Minna E. Jewell and Howard B. Lewis.

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